

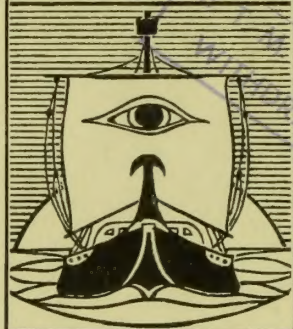
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REPORTS

OF THE

SLEEPING SICKNESS COMMISSION

OF THE

ROYAL SOCIETY.

No. XI.

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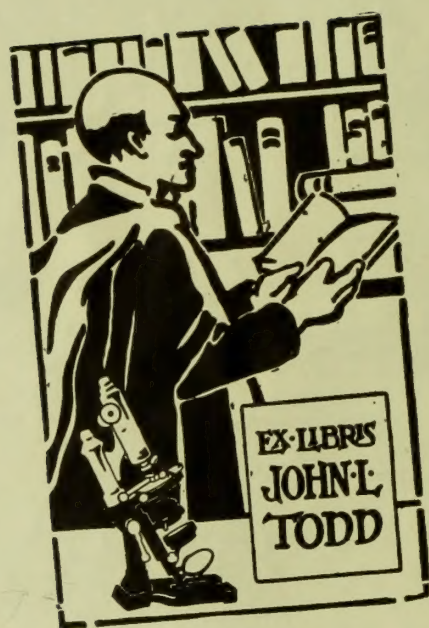
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REPORTS
OF THE
SLEEPING SICKNESS COMMISSION
OF THE
ROYAL SOCIETY.

No. XI.

SLEEPING SICKNESS AND OTHER DISEASES
OF MAN AND ANIMALS IN UGANDA
DURING THE YEARS 1908-9-10.

BY

COLONEL SIR DAVID BRUCE, C.B., F.R.S., A.M.S.;
CAPTAINS A. E. HAMERTON, D.S.O., AND H. R.
BATEMAN, R.A.M.C.; CAPTAIN F. P. MACKIE,
I.M.S.; AND LADY BRUCE, R.R.C.

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INTRODUCTION.

THIS, the third Commission sent out to Uganda since 1902, under the direction of the Royal Society, for the study of Sleeping Sickness, was formed at the suggestion of the late Lord Ripon, then Secretary of State for the Colonies, for the purpose of continuing the investigation and utilising the laboratory built for the Commission in 1906.

The *personnel* of the 1908 Commission was as follows: *Director*, Colonel Sir David Bruce, C.B., F.R.S., A.M.S. *Members*, Captains A. E. Hamerton, D.S.O., and H. R. Bateman, R.A.M.C.; Captain F. P. Mackie, I.M.S.; and Lady Bruce, R.R.C. *Laboratory Assistant*, Staff-Sergeant A. Gibbons, R.A.M.C. *Secretary*, Mr. James Wilson.

The Commission left England on September 16, 1908, and reached Mombasa, British East Africa, on October 14. Here the Commission was received, and the rough places made smooth for it, by Mr. Waller, the Coast Agent to the Uganda Protectorate, and Mr. Stanley, the Assistant Traffic Manager to the Uganda Railway. The Commission was also most hospitably entertained by Dr. Hinde, the Acting Commissioner.

After spending two days at Mombasa waiting for a train, a start was made for the Lake. At Nairobi, the Commission met the Principal Medical Officer (Lieutenant-Colonel J. Will, R.A.M.C.) who, unfortunately, was on the point of leaving the country to return to military duty. Here also the Commission received a telegram from His Excellency Sir J. Hayes Sadler, K.C.B., then Governor of British East Africa, with the information that he would meet the members at Naivasha, higher up the line. At Naivasha the carriage of the Commission was attached to the Governor's special train, and proceeded with him to Port Florence.

From the Governor the Commission heard that Sleeping Sickness had of late been making sad havoc among the Kavirondo on the eastern shores of the Lake, a large percentage of whom were reported to be infected. This seemed to be due to the habit these people had of fishing in the rivers which run into this part of the Lake. These rivers are streams of clear water, with thickly wooded banks, and swarm with tsetse flies.

During the journey up country the Commission had a good view of the snow-capped Kilimanjaro from the Kapiti Plains, and moreover saw on the Athi Plains an enormous number of wild animals, including several giraffe.

On arrival at Entebbe on Monday, October 16, the Commission was most kindly received by Dr. A. D. P. Hodges, C.M.G., Principal Medical Officer; Mr. J. W. P. Russell, Director of Transport; and others of the Uganda Government officials, who had come down to the landing-place to welcome the Governor, who was returning from British East Africa. In Entebbe the Commission remained until Wednesday, October 21.

A great improvement was seen in the Government Cantonment of Entebbe. In 1903, all the houses were thatched with grass,

productive of many fires, there were no glass windows except in the then newly-built Government House, there was no club, no golf course, and no means of conveyance into the native capital of Kampala. Now all the roofs are of corrugated iron, all the houses have windows, there is a still newer Government House, a first-rate club, an excellent golf course, and a motor-bus which runs into Kampala daily.

The Commission remained in Kampala for one night, where they have to thank Captain de Courcy Ireland, King's African Rifles, for his hospitality, and at last reached their destination, Mpumu, on Thursday, October 22.

Here they found the Royal Society's Laboratory and two good houses built, while another was in course of construction. The laboratory and one of the houses had been brought from the spot on the Lake-shore, some three miles from Entebbe, where they had been built in 1906. Previous to that the laboratory had been situated in Entebbe. This camp at Mpumu ($0^{\circ} 15' N.$, $32^{\circ} 50' E.$) is on the top of a flat-topped hill with steep sides, standing some 500 feet above the surrounding country. It is 750 yards long and, roughly, 250 yards broad; its long axis runs in a north-westerly and south-easterly direction. It lies six miles from the Lake at its nearest point—Kibanga, on Buka Bay. The surrounding country is very hilly, Mpumu, about 4,300 feet above sea-level, being on a level with most of the adjacent hills; many of these are wooded, and between the hills the country is swampy.

The Commission shortly after their arrival set up a meteorological station, the instruments being lent by the Botanical, Forestry, and Scientific Department, and observations made during the next twelve months, from November 1, 1908, to October 31, 1909, show a temperature ranging between 60° and 83° F. Mpumu has, therefore, a temperate and pleasant climate. The rainfall is, of course, high: 56 inches fell during the year. The highest monthly rainfall was in November, 1908, when 9.22 inches fell; the lowest in February, 1909—0.20 inch. Details of the meteorological returns are given in Appendix I.

Kampala, the native capital, where supplies were obtained, is 27 miles to the west, and headquarters another 22 or 23 miles further away. The position chosen for the camp was, therefore, isolated and difficult of access, which rendered the work of the Commission more difficult and slower than if a more accessible site had been chosen. In all probability experience will show that a mistake has been made in placing the laboratory in such an isolated place, and it will be found necessary to again remove it and place it within easier reach of Entebbe and Kampala. No doubt the difficulty of choosing a suitable site was great, especially if the principle was followed that the camp must be in the vicinity of a Sleeping Sickness concentration camp as well as the Lake. But the nearest Sleeping Sickness concentration camp was seven miles of steep native paths away, and for all practical purposes might have been seventy.

The Laboratory, 45 feet long and 18 feet wide, is built of corrugated iron. It is capable of accommodating six workers, and is



FIG. 1.
LABORATORY AND OFFICE, MPUMU.



FIG. 2.
MONKEY HOUSE, MPUMU.



FIG. 3.
DIRECTOR'S HOUSE, MPUMU.



FIG. 4.
MESS, MPUMU.



FIG. 5.
THE DESTROYER, MPUMU.



excellently adapted for the work. It is well furnished with all the essentials, and has a supply of pure rain water from the roof, collected in two tanks, each of 400 gallons capacity. The rain-water collected in the pure atmosphere of Mpumu is equal to distilled water, and we used it as such. The photograph (fig. 1) shows the laboratory and the office.

Monkey House.—Fig. 2 is the monkey house. This is a large fly-proof shed, 30 feet square. Monkeys are much healthier and happier in the open air, and as there had been a good number of deaths among the monkeys confined in this house, it was given up in favour of open-air boxes. Experience teaches that the common wild monkey in Uganda will live for years in captivity in perfect health if kept in the open air. As it is sometimes necessary to keep experimental monkeys under observation for months, it is obvious that all closed-up monkey houses are a delusion and a snare. What can be more annoying than to lose the work of months by an experimental monkey dying of some extraneous malady?

Director's House.—This, as can be seen from fig. 3, is a large house, constructed of corrugated iron.

The Mess.—Fig. 4. This and the other houses erected are the ordinary wattle and daub huts, thatched with grass, which are used in Uganda by the better-class natives. They are cool, comfortable, and picturesque, but have one disadvantage—their liability to be set on fire by lightning. There is, it is said, little danger of this in the vicinity of iron-roofed houses, as the latter will probably attract the lightning to a much greater extent.

The hill is formed of ironstone, and violent thunderstorms are common. Several trees on the hill were struck by lightning during the time the Commission were at Mpumu, but fortunately no lives were lost.

The Water Supply.—The natural water supply of Mpumu is a small mud hole, some 5 feet in diameter and 1 foot deep, situated at the foot of the hill to the south-east. This is surrounded by native huts and cultivated ground, and as ankylostomiasis is almost universal among the natives of Uganda, there seemed reasonable ground for looking on this water supply as dangerous, even for bathing purposes. Mr. S. Caink, the District Engineer, however, came to the rescue by digging a cistern to hold 3,000 gallons in the ironstone rock. This, like the widow's cruse, has been full and overflowing ever since with beautifully clear and cool water collected from the iron roof of the Director's house. For every inch of rain which falls 1,300 gallons of water run into the cistern, giving a total of 73,000 gallons during the year, or 30 gallons per head per diem of the white population on the hill. This water supply was gained at small cost and has been an unqualified success.

Conservancy Arrangements.—In order to keep the surroundings of the camp clean, a destructor was built to burn up the rubbish. This was also cut out of the ironstone rock, and consisted, as will be seen from fig. 5, of a chamber some 5 feet square, fed from above through a circular opening 16 inches in diameter.

The front and roof were made out of sheets of corrugated iron, enclosing a thick layer of clay. The chimney, which was also made of corrugated iron sheeting, was 9 feet high. This destructor was also a success, and all refuse from cook-houses, &c., was brought here and burnt, instead of being thrown away among the long grass, and so causing in time an unsightly nuisance.

Cattle Sheds.—As the Commission, in addition to Sleeping Sickness work, undertook the investigation of the various animal trypanosome diseases of Uganda, as well as an important disease among calves called “Amakebe,” three separate cattle sheds and cattle runs were constructed—one for healthy oxen, another for trypanosome disease, and the third for “Amakebe.” In addition, several tick-free stables and yards were built, which proved of great use in the investigation of “Amakebe.” Trypanosome disease was causing a large number of deaths among the Government transport oxen, as well as among the native cattle, and neither its true nature nor its carrier was known. “Amakebe” was said to cause a mortality among calves of from 40 to 60 or more per cent.

Collection of Flies.—As the study of *Glossina palpalis* and its relation to Sleeping Sickness naturally stood in the front rank as a subject of study by the Commission, it was necessary to have a large supply of tsetse flies to work with. The Lake-shore is six miles away, so that it was not possible for the fly-boys to walk up and down every day, and also find time for catching the flies. The six fly-boys, therefore, lived in a hut near the Lake-shore at Kibanga, one of the authorised landing places. This landing place on the arrival of the Commission was found to be in a neglected and overgrown condition, and tsetse flies were quite numerous. As a market at that time was held here once a week, at which some hundreds of natives congregated from Buvuma and other infected islands, to sell earthenware pots and dried fish to the natives on the mainland, the danger of such a state of affairs was unmistakable. This, on being pointed out to the “Sekibobo”—the head Chief of the district—was at once remedied, and afterwards not a single tsetse fly was caught at Kibanga. But as a consequence the fly-boys were forced to use a canoe to reach a spot where flies could be caught. A suitable place was found a short distance away, and the flies are so numerous along the Lake-shore, that although the boys caught about 500 flies daily at the same spot, the supply showed no signs of diminishing.

Historical.—For the information of the lay readers of this Report, it may be useful to summarise the work already done on Sleeping Sickness by the various Commissions sent out to Uganda by the Royal Society. The first was sent out in 1902. The second Commission, sent out in 1903, first discovered that Sleeping Sickness is due to the action of an animal blood parasite—the *Trypanosoma gambiense* (Dutton). It also established the fact that this parasite is conveyed from the sick to the healthy by a species of tsetse fly—the *Glossina palpalis*. The Commission then worked out the distribution of this fly in Uganda, and found that it coincided in a remarkable manner with the distribution of

Sleeping Sickness. This constituted a strong proof that this fly is the only carrier of the disease. No tsetse fly, no Sleeping Sickness. The distribution of the fly is a peculiar one. It is only found at the edge of open water where there is plenty of forest shade. This knowledge rendered it easy for intelligent persons to avoid the disease, by keeping out of the fly area; if it was necessary to pass through it precautions could be taken against being bitten by the fly. It also enabled the Uganda Administration to organise preventive and stamping out measures on a rational basis.

In 1904 the discovery of the useful method of diagnosis by puncturing a gland was discovered.

In 1906 the Commission came to an abrupt end, when one of its members, the late Lieutenant Forbes Tulloch, R.A.M.C., most unfortunately contracted the disease. It had been the intention of the Royal Society to keep this Commission constantly at work, by sending out fresh workers from time to time, until all the important diseases in Uganda, both of men and animals, had been identified and their distribution mapped out. It is only by such knowledge that our tropical Colonies and Protectorates can be expected to progress satisfactorily.

During this time, in addition to the work done by the Commission, the medical officers of the Protectorate have greatly advanced our knowledge of the distribution of Sleeping Sickness and *Glossina palpalis* in Uganda, and also of the treatment of the disease. This has been mainly due to the energy of Dr. R. U. Moffat, C.M.G., and Dr. A. D. P. Hodges, C.M.G., late and present Principal Medical Officers of the Protectorate. Among others, Drs. C. Wiggins, C. J. Baker, A. G. Bagshawe, and R. A. L. van Someren, deserve special mention for the excellent work they have done.

In 1908 the Commission was reconstituted, and it is to be hoped it will now continue in existence until the outstanding problems of disease in Uganda are solved.

The work done by the Commission from October 22, 1908, to the end of July, 1910, is shown in the Table of Contents of this Volume. Some of the papers written by the Commission have already been published in the Reports of the Sleeping Sickness Commission, Volume X., but the titles of these are also given in the Table of Contents of the present volume.

The Director and Lady Bruce left Mpumu to return to England by way of the Nile on December 5, 1909, and arrived in London, February 26, 1910. Captain Mackie left on November 18, 1909, and arrived in England April 14, 1910. His deputation with the Commission ceased on August 19, 1910. Captains Hamerton and Bateman remained at Mpumu until the beginning of August, 1910, when they left for England, arriving in London, *via* Mombasa, on September 18, 1910. Captain A. D. Fraser, R.A.M.C., and Drs. Carpenter and Duke are at present (March, 1911) carrying on the work of the Commission.

One of the chief additions to our knowledge of the etiology of Sleeping Sickness during the last two years is, the discovery by

Professor Kleine, at the end of 1908, of a development of *Trypanosoma brucei* taking place in *Glossina palpalis*. Previous to this it was believed that the parasite was carried by the fly from the sick to the healthy in a mechanical way, just as vaccine would be conveyed from one arm to another on the point of a lancet. Now it is found that this only occurs exceptionally, and that, as a rule, the fly passes on the parasite after a period of development of some thirty days. Then, it was believed that the fly could only remain infective for 48 hours; now, that when once infective it remains so for the rest of its life, which is probably a fairly long one.

Another addition to our knowledge is that the tsetse flies on the Lake-shore still remain infective years after the natives have been removed. This points to there being some reservoir of the virus other than man from which the fly may infect itself.

Still another interesting fact discovered in 1909 was that Malta fever prevailed to a large extent among the natives living near Lake Albert Edward, and that it was due to the drinking of goats' milk, just as had been proved to be the case in Malta.

The mystery which has hitherto attached to that fatal disease of calves—"Amakebe"—was also cleared up. The disease was found to be none other than the so-called East Coast fever, which has played such havoc of late years in South Africa.

Large collections of the biting flies and ticks in Uganda were made, and an attempt is made here to describe their distribution.

The ground is also broken in regard to the distribution of cattle diseases in Uganda.

Six appendices are added giving meteorological observations, reports on various outbreaks of disease among cattle, &c.

The members of the Commission take this opportunity of expressing their most sincere thanks, in the first place, to Sir Apolo Kagwa, K.C.M.G., the Prime Minister of Uganda, for the very great assistance he at all times gave to the Commission. Without his aid much less would have been accomplished. Sir Apolo arranged for cases of "Muhinyo" to be sent from the distant Lake Albert Edward to Mpumu for the study of that disease. When this was found to be Malta fever, he had a herd of goats from the infected area also brought to the laboratory. He supplied the Commission with several cows about to calve, in order that the Commission might have susceptible animals for the study of "Amakebe," and sent from his estates numerous examples of cattle suffering from this and other diseases. In short, there was no trouble too great which he did not willingly take to forward the aims of the Commission.

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In conclusion, the Commission would desire to express its appreciation of the assistance rendered to it by Staff-Sergeant A. Gibbons, R.A.M.C., and Mr. James Wilson in the work at Mpumu. Sergeant Gibbons was unceasing in activity from early morning till late at night. He was draughtsman, architect, carpenter, builder, microscopist, commissariat officer, and many other professions and trades in turn. Whenever any difficulty arose at any time, his assistance was at once sought, and the Commission cannot express strongly enough its appreciation of his services. Mr. Wilson also proved himself invaluable. He had charge of the correspondence, which on account of the isolated position of Mpumu, tended to become voluminous. In addition he was bookkeeper, entering all observations from each worker's day-book into the ledger, and arranging, classifying, collating, and tabulating the work as it proceeded, so that at any time a clear view could be had of the progress of any particular line of investigation. He was also paymaster and took charge of the accounts. As there were usually more than a hundred natives employed in various ways, the keeping of the pay-book was no easy matter. In all this he was most persevering and untiring, and the Commission owe him also a deep debt of gratitude for his intelligent and loyal services.

A. TRYPANOSOMA GAMBIENSE AND GLOSSINA PALPALIS.

1.—THE DEVELOPMENT OF TRYPANOSOMA GAMBIENSE IN GLOSSINA PALPALIS.

This paper has already been published in the 'Proceedings' of the Royal Society, B, Vol. 81, 1909, p. 405, and reprinted in the Reports of the Sleeping Sickness Commission, Vol. X., p. 46. It described a single experiment in which late development of *Trypanosoma gambiense* took place in *Glossina palpalis*. An attempt was also made to state the proportion of infected flies to non-infected in Nature.

2.—THE DEVELOPMENT OF TRYPANOSOMES IN TSETSE FLIES.*

In the 'Proceedings' of the Royal Society (B, Vol. 81, 1909) a paper was published describing a single experiment illustrating the development of *Trypanosoma gambiense* in *Glossina palpalis*.† This experiment was carried out at Mpumu, Uganda, near Lake Victoria, in the spring of 1909. Since that date many experiments, on the same lines, have been made, not only with *Trypanosoma gambiense* but also with *Trypanosoma pecorum*, *vivax*, *nanum*, *brucei* and *uniforme*. It is proposed to describe these further experiments in this paper.

It will be remembered that Kleine, in German East Africa, at the end of 1908, made the discovery that *Glossina palpalis* could convey *Trypanosoma brucei* for some 50 days after the fly had fed on an infected animal. Following Kleine's lead, our experiments were carried out, at first with Lake-shore flies, afterwards with flies bred in the laboratory.

(a.)—*The Development of Trypanosoma gambiense in Glossina palpalis caught on the Lake-shore.*

These experiments were carried out with ordinary wild tsetse flies caught on the Lake-shore, and therefore open to the doubt that some of them may have been naturally infected when they were captured. As there is some evidence that one fly in 400 or 500 of the wild Lake-shore flies is found to be naturally infected, it is evident that these previously infected flies may lead into error. It will be seen later that this risk is not run when flies bred in the laboratory are used.

The flies when brought up from the Lake-shore were kept in small boxes, with mosquito-netting sides, and placed over dishes containing water, to imitate, as far as possible, their natural conditions. It may be remarked here that these tsetse flies are so numerous on the shores of Victoria Nyanza, and the supply so

* Reprinted with additions from the 'Proceedings' of the Royal Society, B, Vol 82, 1910.

† Also Reports of the Sleeping Sickness Commission, No. X., p. 46.

unending, that the fly-boys brought up some 500 every day, and these usually caught at only one or two spots.

The method of carrying out these experiments was always the same. The flies were fed for some days on a highly infected monkey, whose blood on microscopical examination was seen to contain numerous trypanosomes of Sleeping Sickness, and afterwards on a series of healthy monkeys.

The following table gives the number of flies in each experiment, the number of days they were fed on a monkey whose blood contained *Trypanosoma gambiense*, the number of days which elapsed before the flies became infective, and the number of days the flies remained infective. The minus signs signify that the flies failed to become infected, or at least failed to infect; or, in other words, that the experiment was negative.

Table 1.—Development of *Trypanosoma gambiense* in Lake-shore *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
624	60	3	—	—
656	280	3	—	—
663	60	2	18	75
676	500	3	29	47
721	50	3	—	—
980	350	3	—	—
986	100	2	19	35
987	50	2	40	51
989	50	2	45	45
1020	100	3	—	—
1023	50	3	37	51
1026	70	3	34	48
1198	20	40	—	—
1372	100	3	—	—

Of these 14 experiments, seven are positive and seven negative. In the positive experiments 880 flies were used, an average of 126; in the negative 960, an average of 137. The shortest time which elapsed before a fly became infective was 18 days, the longest 45 days, and the average 32 days.

It may be well to give some of these experiments more in detail, in order to show the methods used, and draw attention to various interesting points.

Experiment 624.

To ascertain if development of Trypanosoma gambiense takes place in the alimentary canal of Wild or Lake-shore Glossina palpalis.

March 31, 1909.—Two batches of *Glossina palpalis*, caught on the Lake-shore, consisting of 30 flies in each batch, were fed to-day on a monkey whose blood contained numbers of *Trypanosoma gambiense*.

The following table gives the principal details of the experiment:—

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1909. Mar. 31	—	Flies fed on infected monkey.			
Apr. 1	1	" "			
" 2	2	" "			
" 3—4	3—4	Flies starved 72 hours.			
" 5—8	5—8	Flies fed on healthy monkey, 657		—	
" 9—12	9—12	Flies fed on healthy monkey, 677		—	
" 13	13	Flies fed on healthy monkey, 702		—	
" 14	14	Flies fed on healthy monkey, 703		—	
" 15	15	Flies fed on healthy monkey, 704		—	
" 16	16	Flies fed on healthy monkey, 705		—	
" 17—21	17—21	Flies fed on healthy monkey, 706		—	
" 22— May 22	22—52	Flies fed on healthy monkey, 728		—	

Remarks.—These 60 Lake-shore or wild flies, although fed on an infected monkey for three days, failed to convey the infection to healthy monkeys. As the flies died they were dissected. Only in one were flagellates found, and these appeared to be of the *Trypanosoma grayi* type. An emulsion was made of the contents of the alimentary canal of this fly and injected into monkey 914. Monkey 914 never showed trypanosomes in its blood, although kept under observation for a month.

Experiment 656.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1909. Apr. 3—5	1—2	280 flies fed on infected monkey.			
" 6—7	3—4	Flies starved 72 hours.			
" 8—17	5—14	Flies fed on healthy monkey, 675		—	
" 18	15	Flies fed on healthy monkey, 712		—	
" 19	16	Flies fed on healthy monkey, 713		—	
" 20	17	Flies fed on healthy monkey, 714		—	
" 21	18	Flies fed on healthy monkey, 715		—	
" 22—25	19—22	Flies fed on healthy monkey, 716		—	
" 26—28	23—25	Flies starved.			
" 29— June 17	26—75	Flies fed on healthy monkey, 744		—	June 8, 8 flies alive June 18, 2 flies alive.

Remarks.—Two hundred and eighty wild tsetse flies, fed for three days on an infected monkey and then on healthy monkeys, failed to transfer the disease. The experiment lasted from April 3 to June 17, and seven healthy monkeys were used. After 66 days eight flies remained alive; after 75 days only two. None of the flies which died, or were killed and dissected, showed flagellates in the alimentary canal.

Experiment 663.

This is the experiment described at length in the 'Proceedings' of the Royal Society (B, vol. 81, 1909). Sixty wild flies were used. One fly became infective after 18 days, and remained infective 75 days, when it died. A small quantity of fluid from the gut of this fly injected into a healthy monkey gave rise to Sleeping Sickness.

Experiment 676.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Apr. 6—8	1—2	500 Lake-shore flies fed on infected monkey.			
„ 9—10	3—4	Flies starved.			
„ 11—16	5—10	Flies fed on healthy monkey, 696		—	
„ 17—18	11—12	Flies fed on healthy monkey, 707		—	
„ 19—20	13—14	Flies fed on healthy monkey, 708		—	
„ 21	15	Flies fed on healthy monkey, 709		—	
„ 22	16	Flies fed on healthy monkey, 710		—	
„ 23—	17—36	Flies fed on healthy monkey, 711	+		April 23, 67 flies alive.
May 12					
May 13—14	37—38	Flies starved.			
„ 15—16	39—40	Flies fed on healthy monkey, 766	+		
„ 17—21	41—45	Flies fed on healthy monkey, 770	+		
„ 22—23	46—47	Flies starved ...			
„ 24—28	48—52	Flies fed on healthy monkey, 901		—	May 23, 22 flies alive. Infected fly found.
„ 29—30	53—54	Flies starved.			
„ 31—	55—58	Flies fed on healthy monkey, 941		—	June 3, 1 fly alive.
June 3					

Remarks.—Five hundred Lake-shore tsetse flies were fed for three days on an infected monkey. As none of the five healthy monkeys on which these flies were fed during the first 16 days showed any sign of Sleeping Sickness it may be assumed that there was no naturally-infected fly among the 500. On or about the 29th day the cage of flies became infective, and remained infective up to the 47th day. On this day a dead fly was found on dissection to contain flagellates, and after the death of this fly no further infection took place. The injection of the infected

fly failed, however, to give rise to Sleeping Sickness when injected under the skin of a healthy monkey.

Experiment 721.

Remarks.—This experiment lasted from April 12 to July 6, a period of 85 days, and remained negative throughout. On the 30th day, 12 flies out of the 50 remained alive; on the 73rd day only six flies were left. As the flies died they were dissected, but no infected fly was found.

Experiment 980.

Remarks.—This experiment lasted 66 days, and remained negative. At the end, 15 flies remained alive. These were killed and dissected. All proved negative.

These experiments on the development of *Trypanosoma gambiense* in Lake-shore or wild *Glossina palpalis*, given somewhat in detail, will suffice to show the method employed, and make it unnecessary to explain the remaining experiments further than is done in Table 1.

It would appear from the fact that none of the healthy monkeys became infected before the 18th day, that not a single fly of the 1,840 used was infective when captured. That is to say, that among nearly 2,000 Lake-shore flies, not one was naturally infected. On referring to Table IV., in the previous paper in the 'Proceedings' of the Royal Society*—a table showing the probable number of naturally-infected flies—this is seen to be by no means exceptional.

Other points of interest arising out of these experiments are the number of flies which became infective, and the result of injecting their body-contents into healthy animals.

The following table shows this:—

Table 2.—Number of Flies found Infected with Trypanosomes in the Experiments with Lake-shore Flies and *Trypanosoma gambiense*.

Experiment.	No. of flies used.	Experiment, positive, or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
624	60	—	1	Negative ...	<i>T. grayi</i> type.
656	280	—	0		
663	60	+	1	Positive.	
676	500	+	1	Negative.	
721	50	—	0		1 <i>T. gambiense</i> and 1 <i>T. grayi</i> type.
980	350	—	0		
986	100	+	1	Not injected.	
987	50	+	2	Negative ...	
989	50	+	1	Negative.	
1020	100	—	0		
1023	50	+	5	2 positive. 3 negative.	
1026	70	+	4	3 positive. 1 negative	
1198	20	—	0		
1372	100	—	0		

* B, Vol. 81, 1909, p. 413; also Reports of the Sleeping Sickness Commission, No. X., p. 53.

Thus it is seen that the infected flies found in three of the positive experiments when injected into healthy monkeys gave negative results, while those found in three others gave positive results. The infected fly found in the seventh successful experiment was not injected.

It is difficult to understand why the results of injecting infected flies into healthy animals are so irregular. The only theory brought forward is that the trypanosomes introduced under the skin along with the tissues of the fly may give rise to a reaction at that point, which will so damage the parasites that they fail to infect.

In these experiments, 1,840 flies were used, and of these 16 became infected, or, at least, were found to have flagellates in their gut. This works out at less than 1 per cent. The smallness of the percentage is due to the fact that less care was taken to dissect the flies which died during the course of the experiments.

(b.)—*The Development of Trypanosoma gambiense in Laboratory-Bred Glossina palpalis.**

The pupæ of the fly were found on the Lake-shore, and hatched out in the laboratory. For a long time the Commission failed to find any pupæ, although days were spent in turning over soil and decaying vegetable matter in those places where the fly most abounded. At last, Captain A. D. Fraser, Royal Army Medical Corps, found them in numbers in patches of sand on the edge of the Lake in the Sesse Islands. After the Sesse Islands were emptied of their inhabitants, Fraser's native collectors came into the service of the Commission, and from that time there was no lack of pupæ. These natives found them in large numbers. One day they brought up as many as 7,000. These pupæ proved to be much healthier than those obtained from flies in captivity. The flies bred from larvæ born in the laboratory rarely showed any marked vitality. Many of the larvæ were immature, and those which hatched out were rarely as successful as experimental flies. On the other hand, the flies hatched out from pupæ found on the Lake-shore were fairly strong and vigorous, and lived in captivity for a couple of months or more. It was, however, difficult to get them to feed at first, and very few became infective, as the following table shows. The flies were fed chiefly on infected monkeys. In one negative experiment (1431) they were fed on a case of Sleeping Sickness in man, and in five—two positive (1566 and 1602) and three negative (1269, 1452, and 1672)—on oxen. Numerous observations went to show that there is no hereditary transmission of trypanosomes in *Glossina palpalis*; and no evidence was gained that the flies become infected with any flagellate by contact with other flies or fouled cages. Any trypanosomes found in laboratory-bred flies may therefore be considered to be derived from the infected animal they had fed upon.

* Further experiments on this subject are published in the 'Proceedings' of the Royal Society, B, Vol. 83, 1911, p. 513; and at p. 34 of this Report.

Table 3.—Development of *Trypanosoma gambiense* in Laboratory-Bred *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
725	8	3	—	—
733	8	3	—	—
750	15	6	—	—
768	9	5	—	—
858	9	8	—	—
915	6	10	—	—
947	9	5	—	—
975	16	5	34	49
991	27	6	—	—
1266	11	12	—	—
1269	22	7	—	—
1368	14	12	—	—
1397	7	19	—	—
1428	50	6	—	—
1431	30	20	—	—
1452	90	10	—	—
1549	50	4	37	46
1558	60	4	—	—
1559	50	4	—	—
1566	35	4	53	53
1602	50	4	27	27
1604	35	12	—	—
1640	40	10	—	—
1651	60	4	41	41
1664	50	12	—	—
1665	50	12	—	—
1671	45	12	—	—
1672	28	12	—	—
1680	45	4	—	—
1686	60	3	—	—
1688	60	3	—	—
1693	50	4	—	—
1706	60	7	—	—
1712	50	12	32	32
1758	50	3	—	—
1760	60	4	29	29
1761	50	4	38	38
1769	60	3	—	—
1801	70	12	—	—
1802	75	12	—	—
1860	60	12	—	—
1868	60	12	—	—

Among these 42 experiments there were only eight positive, while there are as many as 34 negative. In the eight positive experiments, 371 flies were used, an average of 46; in the 34 negative experiments, 1,323 flies, an average of 40. The shortest time which elapsed before a laboratory-bred fly became infected with *Trypanosoma gambiense* was 27 days, the longest 53 days, and the average 36 days.

Here follow the experiments, given somewhat more in detail, which gave positive results:—

Experiment 975.

To ascertain if any development of Trypanosoma gambiense takes place in the alimentary canal of laboratory-bred Glossina palpalis.

The following table gives the principal details of the experiment:—

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1909. June 8—12	1—4	Flies fed on infected monkey.			16 flies used.
„ 13—15	5—7	Flies starved.			
„ 16—	8—41	Flies fed on healthy monkey, 1008	+		
„ July 19					
July 20—21	42—43	Flies starved.			Aug. 4, 12 remain- ing flies killed and dissected.
„ 22—	44—55	Flies fed on healthy monkey, 1374	+		
„ Aug. 3					

Remarks.—Sixteen laboratory-bred flies were fed on infected monkeys for five days. Thirty-four days after their first feed they became infective, and remained so for at least 49 days. On the 56th day the remaining 12 flies were dissected and examined for flagellates. None were found, but the contents of the alimentary canals of the 12 flies, pooled and injected into a healthy monkey, gave rise to Sleeping Sickness.

Experiment 1549.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1909. Aug. 23—27	1—4	Flies fed on heavily-infected monkey.			50 flies used.
„ 28—29	5—6	Flies starved.			
„ 30—	7—43	Flies fed on healthy monkey, 1617	+		Oct. 8, 20 remain- ing flies dissected, 4 found infected.
„ Oct. 5					
Oct. 6—7	44—45	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed on an infected monkey daily for five days. Thirty-seven days after their first feed they became infective. Twenty flies remained alive on the 46th day, and on dissection four were found infected with flagellates. A drop of fluid from the alimentary canals of two of these infected flies injected into a monkey gave rise to Sleeping Sickness.

Experiment 1566.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Aug. 29—	1—3	Flies fed daily on infected ox	+		35 flies used.
Sept. 1		Flies starved.			
Sept. 2—3	4—5	Flies fed on healthy monkey, 1566			Oct. 21, 20 remaining flies killed and dissected ;
" 4—	6—51	Flies starved.			9 found infected.
Oct. 19					
Oct. 20	52				

Remarks.—Thirty-five laboratory-bred flies were fed daily for four days on an ox infected with *Trypanosoma gambiense*. Fifty-three days after their first feed the flies became infective. Nine of the 20 remaining flies, when dissected, showed infection with flagellates: three of these had infection of the proboscis. The contents of the alimentary canal of one infected fly injected into a monkey and goat gave negative results, as also did the probosces of two infected flies when injected into a goat.

Experiment 1602.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Sept. 1—4	1—3	Flies fed on infected ox daily			50 flies used.
" 5—6	4—5	Flies starved.			
" 7—14	6—13	Flies fed on healthy monkey, 1620		—	
" 15—20	14—19	Flies fed alternately, daily, on healthy monkey, 1620, and goat, 1690		—	
" 21—29	20—28	Flies fed alternately, daily, on healthy monkey, 1703, and goat, 1690		1703 + 1690 —	Sept. 29, 32 remaining flies killed by accident and dissected ; 5 found infected.

Remarks.—Fifty laboratory-bred flies were fed daily for four days on an infected ox, as in the previous experiment. From the 15th to the 28th day the flies were fed alternately on a monkey and goat. Twenty-seven days after their first feed the flies infected monkey 1703 with Sleeping Sickness. Five of the 32 remaining flies showed infection with flagellates, and one of these injected into a monkey gave rise to Sleeping Sickness.

Experiment 1651.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Sept. 11—14	1—3	Flies fed on infected monkey			60 flies used.
„ 15—16	4—5	Flies starved.			Monkey 1651 died on 13th day.
„ 17—24	6—13	Flies fed on healthy monkey, 1651		—	Nov. 1, 32 remaining flies killed and dissected ;
„ 25— Oct. 29	14—48	Flies fed on healthy monkey, 1720	+		4 found infected.
Oct. 30—31	49—50	Flies starved.			

Remarks.—Sixty laboratory-bred flies were fed daily for four days on an infected monkey. Forty-one days after their first infected feed they became infective. Four flies infected with flagellates were found among the 32 which remained alive on the 51st day. They were not injected into animals.

Experiment 1712.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Sept. 23— Oct. 5	1—12	Flies fed on infected monkey			50 flies used.
Oct. 6—7	13—14	Flies starved.			Nov. 4, 31 remaining flies killed and dissected ;
„ 8— Nov. 2	15—40	Flies fed on healthy monkey, 1790	+		1 found infected.
Nov. 3	41	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed daily for 13 days on an infected monkey. The flies became infective 32 days after their first feed. On dissection of the remaining 31 flies on the 41st day, one was found to be infected with flagellates, but it did not give rise to the disease when injected into a healthy monkey.

Experiment 1760.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Oct. 1—5	1—4	Flies fed on infected monkey			60 flies used.
„ 6—7	5—6	Flies starved.			Nov. 4, 1 infected fly injected into healthy monkey.
„ 8— Nov. 6	7—36	Flies fed on healthy monkey, 1760	+		Nov. 8, 28 remaining flies killed and dissected ; all negative.
Nov. 7	37	Flies starved.			

Remarks.—Sixty laboratory-bred flies were fed daily for five days on an infected monkey. They became infective on the 29th day. On the 34th day one fly which had died was found to be infected with flagellates, but on injection into a monkey it failed to give rise to Sleeping Sickness. No infected flies were found among the 28 remaining alive on the 38th day, when they were dissected.

Experiment 1761.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Oct. 1—5	1—4	Flies fed on infected monkey			50 flies used.
" 6—7	5—6	Flies starved.			
" 8— Nov. 8	7—38	Flies fed on healthy monkey, 1761	+		Nov. 11, 31 remaining flies killed and dissected; all negative.
Nov. 9—10	39—40	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed daily for five days on an infected monkey. They became infective on the 38th day. No infected flies were found among the 31 remaining alive on the 41st day, when they were dissected.

Table 4.—Number of flies found infected with *Trypanosoma gambiense* in the Experiments with Laboratory-Bred Flies.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
975	16	+	0	Positive ...	12 remaining flies pooled.
1269	22	—	2	Negative.	
1452	90	—	1	"	
1549	50	+	4	Positive.	
1566	35	+	9	Negative ...	1 fly injected.
1602	50	+	5	Positive.	
1651	60	+	4		Flies not injected.
1672	28	—	2	Negative.	
1680	45	—	1	"	
1693	50	—	2	"	
1706	60	—	4	"	
1712	50	+	1	"	
1760	60	+	1	"	
1769	60	—	2	Positive.	
1801	70	—	1		Fly not injected

There are some curious results to be noted here. In Experiment 975 the twelve remaining flies were dissected and examined. None was found infected. They were then pooled and injected

into a healthy monkey, which became infected with Sleeping Sickness. This shows that the infected fly may escape detection by the microscope.

In Experiments 1269, 1452, 1672, 1680, 1693, and 1706, flies were found containing flagellates. These flies had not given rise to disease in the monkey they had been fed on, nor did their injection prove successful. The flagellates must be considered to be *Trypanosoma gambiense*, and therefore a cage of flies may become infected without causing disease either by biting or injection.

In Experiments 1549 and 1602, flies were found containing flagellates, and these flies succeeded in infecting monkeys both by biting while alive and injection after death.

Lastly, in Experiments 1566, 1712, and 1760, flies were found with flagellates which had infected the monkey fed on, but which failed to give rise to disease when their body-contents were injected into healthy animals.

In these experiments 746 laboratory-bred flies were used. Thirty-nine became infected—that is to say, more than 5 per cent.

(c.)—*The Development of Trypanosoma pecorum in Lake-shore Glossina palpalis.*

This is the commonest cattle trypanosome in Uganda. During 1909 it caused epidemics among the Government transport oxen at Entebbe, Mr. Walsh's cattle at Kabula Muliro, and the Uganda Company's cattle at Namukekera, all of which were investigated by the Commission.*

Trypanosoma pecorum belongs to the short, stumpy type of trypanosomes, without free flagella, and is probably identical with that found in Zanzibar by Edington; in Portuguese East Africa, described by Theiler; in Northern Rhodesia by Montgomery and Kinghorn; and in Southern Rhodesia by Bevan.†

Table 5.—Development of *Trypanosoma pecorum* in Lake-shore *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
574	500	3	22	21
996	100	3	—	—
1010	120	3	—	—
1022	100	3	—	—

Only one experiment out of four was successful. The flies became infective 21 days after their first feed on the infected dog.

* See Appendices II and III for reports.

† *Trypanosoma pecorum* is described in the 'Proceedings' of the Royal Society, B, Vol. 82, 1910, p. 468; also at p. 128 of this Report.

Experiment 574.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1909. Mar. 4—6	1—2	Flies fed on infected dog.	+		500 flies used.
" 7—25	3—21	Flies fed on a fowl.			
" 26—30	22—26	Flies fed on healthy monkey, 649			March 25, 120 flies alive.
" 31— Apr. 3	27—30	Flies fed on healthy monkey, 650		—	Apr. 14, 60 flies alive.
Apr. 4—8	31—35	Flies fed on healthy monkey, 660		—	May 3, 30 flies alive.
" 9—13	36—40	Flies fed on healthy monkey, 678		—	May 14, 22 remaining flies dissected; all negative.
" 14— May 13	41—70	Flies fed on healthy monkey, 723		—	

Remarks.—Five hundred wild flies were fed for three days on a dog whose blood contained numerous *Trypanosoma pecorum*. Twenty-one days after their first feed they became infective. By the 27th day they lost the infection and did not regain it, although kept under observation for 71 days. None of the flies which died or were killed and dissected showed any flagellates. It appears as if the infected fly had died early in the experiment and had escaped notice.

Experiment 574 appears to be fairly free from fallacy, and from it, it would seem probable that *Trypanosoma pecorum* can develop in *Glossina palpalis* and infect a healthy animal after a period of 21 days.

(d.)—*The Development of Trypanosoma pecorum in Laboratory-Bred Glossina palpalis.*

Table 6.—Development of *Trypanosoma pecorum* in Laboratory-Bred *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1642	50	4	14	14
1675	50	3	—	—
1676	50	3	—	—
1843	140	4	—	—

Four experiments were carried out, as in the Lake-shore group, and one also was successful. The flies became infective on or about the 14th day.

Experiment 1642.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1909. Sept. 8—11	1—3	Flies fed on infected oxen			50 flies used.
„ 12—13	4—5	Flies starved.			
„ 14—28	6—20	Flies fed on healthy ox, 870.	+		
„ 29	21	Flies starved.			
„ 30— Oct. 11	22—23	Flies fed on healthy monkey, 1741		—	Oct. 13, 35 remaining flies dissected; all negative.
Oct. 12	34	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed on two infected oxen for four days, and then on a healthy ox. Fourteen days after their first infected feed this ox took the disease.

Table 7.—Number of flies found infected with *Trypanosoma pecorum* in the experiments with laboratory-bred flies.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
1675	50	—	1	Negative.	
1827	100	?	1	Negative.	
1843	140	—	2	Negative.	

In reviewing the above experiment with laboratory-bred flies and *Trypanosoma pecorum*, we must bear in mind that Experiment 1642, in which *Trypanosoma pecorum* appears to have been transmitted from an infected to a healthy ox by means of *Glossina palpalis*, is open to fallacy, in that an outbreak of *Trypanosoma pecorum* disease occurred amongst the Commission's cattle about the time this supposed healthy ox showed the disease. It is therefore possible that the animal was accidentally infected. This suspicion is supported by the fact that the flies failed to infect a second healthy animal (monkey), and that no infected flies were found on dissection.

Experiments 1675 and 1676 are open to the objection that the laboratory-bred flies were fed upon infected oxen before feeding on healthy monkeys. We now know that oxen naturally harbour *Trypanosoma gambiense* or *Trypanosoma vivax* in such scanty numbers as to escape detection. We cannot therefore be sure that the trypanosomes in the gut of the infected fly found in Experiment 1675 were developing forms of *Trypanosoma gambiense* and not *Trypanosoma pecorum*, since the injection of the gut of this fly caused no infection.

Experiment 1827 seems to be free from fallacy, but is incomplete, in that we have no record of the healthy animal on which the flies were fed after feeding on the infected monkey.

Experiment 1843, however, is apparently free from all fallacy. Here the flies got their infection from a monkey which was known to be infected with no other trypanosome except *Trypanosoma pecorum*. The flies were subsequently fed upon a healthy monkey for 22 days. The healthy monkey did not become infected, although out of 31 flies dissected 2 were found to contain large numbers of trypanosomes within their intestines. These parasites could scarcely be anything else than a growth of *Trypanosoma pecorum*, although attempts to prove them by subcutaneous inoculation into a susceptible animal produced no result.

In these experiments 290 laboratory-bred flies were used, of which 126 were dissected. Four were found infected—more than 3 per cent.

From the evidence of these experiments we believe that *Trypanosoma pecorum* can develop in *Glossina palpalis*, but we have not sufficient proof that it regains its virulence during its period of development within the fly, since Experiment 574, with wild flies, was not confirmed when repeated with laboratory-bred flies. We are of opinion that *Glossina palpalis* is not the common or exclusive carrier of *Trypanosoma pecorum*, because outbreaks of cattle sickness due to this parasite often occur in *palpalis*-free areas.

(e.)—*The Development of Trypanosoma vivax in Lake-shore Glossina palpalis.*

Table 8.—Development of *Trypanosoma vivax* in Lake-shore *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
997	60	4	11	48
998	45	4	11	44
1014	200	4	21	60 ?

As *Trypanosoma vivax* does not affect monkeys, naturally cattle or goats were used in these experiments. The three experiments with Lake-shore flies were all successful; two became infected in 11 days, and one in 21 days.

Here follow the experiments in detail :—

Experiment 997.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
June 15—18	1—3	Flies fed on infected goat			60 flies used.
" 19—20	4—5	Flies starved.			
" 21—	6—18	Flies fed on healthy calf, 1030.	+		July 6, injected infected fly into goat; negative.
July 3					Aug. 10, remaining flies dissected
July 4—5	19—20	Flies starved.			Infected fly injected into goat; negative.
" 6—21	21—36	Flies fed on healthy bull, 1268.	+		
" 22—23	37—38	Flies starved.			
" 24—	39—56	Flies fed on healthy calf, 737.	+		
Aug. 10					

Remarks.—Sixty wild flies were fed for four days on a goat infected with *Trypanosoma vivax*. Eleven days after their first feed they became infective, and remained so during the experiment. Two flies were found infected with flagellates, one on the 21st day, and one on the 56th day, both of which when injected into goats failed to give rise to the disease.

Experiment 998.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. June 15—18	1—3	Flies fed on infected goat.			45 flies used.
" 19—20	4—5	Flies starved.			
" 21—	6—18	Flies fed on healthy calf, 1030.	+		Aug. 6, pooled contents of 14 flies remaining; injected into goat,
July 3					which became infected with <i>T. vivax</i> .
July 4—5	19—20	Flies starved			
" 6—	21—51	Flies fed on healthy calf, 1267.	+		
" Aug. 5					

Remarks.—Forty-five wild flies were fed for four days on an infected goat. Eleven days after their first feed they became infective, and remained so during the experiment. The pooled contents of the 14 remaining flies injected into a goat on the fifty-second day gave rise to infection with *Trypanosoma vivax*.

Experiment 1014.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. June 18—22	1—4	Flies fed on infected goat.			200 flies used.
" 23—24	5—6	Flies starved.			
" 25—	7—29	Flies fed on healthy goat, 1079	+		July 26, injected infected fly into a goat and monkey, the former of which became infected.
July 17					
July 18	30	Flies starved.			
" 19—	31—46	Flies fed on healthy goat, 1344.	+		Aug. 4, 17 remaining flies dissected; all negative.
" Aug. 3					

Remarks.—Two hundred wild flies were fed for five days on an infected goat. Twenty-one days after their first feed they

became infective, and remained so during the experiment. On the 38th day one infected fly was found, which on injection into a goat and a monkey gave rise to *Trypanosoma vivax* infection in the former animal. Seventeen flies remained alive at the end of the experiment, and were killed and dissected. None of them was found to harbour flagellates.

Table 9.—Number of flies found infected with Trypanosomes in the Experiments with Lake-shore Flies and *Trypanosoma vivax*.

Experiment.	No. of flies used.	Experiment, positive, or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
997	60	+	2	—	14 flies remaining pooled.
998	45	+	2	+	
1014	200	+	1	+	

Of the above three positive experiments it is seen that five infected flies were found. One of these—Experiment 1014—when injected into a susceptible animal gave rise to *Trypanosoma vivax* infection. In Experiment 998, among the 14 remaining flies, two were found with trypanosomes in their probosces. None of the 14 showed flagellates in the gut. The body-contents of the 14 flies, in addition to the contents of the two probosces, were pooled and injected into a goat, which 12 days afterwards showed *Trypanosoma vivax* in its blood.

(f.)—*The Development of Trypanosoma vivax in Laboratory-Bred Glossina palpalis.**

Table 10.—Development of *Trypanosoma vivax* in Laboratory-Bred *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1591	50	4	21	21
1638	25	6	—	—
1698	68	4	35	35
1700	60	4	30	30
1870	50	?	—	—

Five experiments were made with laboratory-bred flies. Three were successful.

* Further experiments on this subject are published at page 50 of this Report.

Experiment 1591.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 2—4	1—2	Flies fed on infected calf, 1318.			50 flies used.
" 5—6	3—4	Flies starved.			
" 7	5	Flies fed on infected calf, 1318.			
" 8—9	6—7	Flies starved.			
" 10—30	8—28	Flies fed on healthy goat, 1652.	+		Oct. 4, 35 remaining flies dissected; negative except 3, which had infected probosces.
Oct. 1—3	29—31	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed for four days on an infected calf; 21 days after their first feed they became infective; 35 flies remained alive on the 32nd day. On these being dissected three were found with infected probosces. These probosces were not injected into animals, so that it is not known if the flagellates were infective by injection.

Experiment 1638.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 6—11	1—5	Flies fed on infected calf.			25 flies used.
" 12—13	6—7	Flies starved.			Sept. 29, 23 remaining flies accidentally killed; negative on dissection.
" 14—29	8—23	Flies fed on healthy goat, 1682.		—	

Remarks.—Twenty-five laboratory-bred flies were fed for six days on a calf infected with *Trypanosoma vivax*, and afterwards for sixteen days on a healthy goat. This goat was not infected, and the remaining 23 flies when killed and dissected all proved negative.

Experiment 1698.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 21—24	1—3	Flies fed on infected ox.			68 flies used.
" 25—26	4—5	Flies starved.			Calf 1893 died 29.11.09.
" 27—	6—45	Flies fed on healthy ox, 425.	+		Nov. 12, 53 flies remaining alive dissected; probosces of 5 swarming with flagellates.
Nov. 6—9	46—49	Flies fed on healthy calf, 1893.		—	
Nov. 10—11	50—51	Flies starved.			

Remarks.—Sixty-eight laboratory-bred flies were fed for four days on an ox whose blood contained *Trypanosoma vivax*. About the 35th day the flies became infective. On the 52nd day the 53 flies which remained alive were killed and dissected. The probosces of five, three males and two females, were found to be swarming with flagellates. These were not injected into animals.

Experiment 1700.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Sept. 21—24	1—3	Flies fed on infected ox.			60 flies used.
" 25—26	4—5	Flies starved.			
" 27—		Flies fed on healthy calf, 290.	+		Oct. 30, 38 remaining flies dissected; 22 found infected.
" Oct. 28	6—37				

Remarks.—Sixty laboratory-bred flies were fed for four days on an ox whose blood contained *Trypanosoma vivax*. About the 30th day these flies became infective. On the 39th day the 38 remaining flies were killed and dissected. There were 19 males and 19 females; 22 showed infection of the proboscis with crithidia-like flagellates. Only one fly showed flagellates in the gut. Of the 22 flies which had trypanosomes in the proboscis, 9 were males and 13 were females; 10 of the infected probosces were ground up in salt solution and injected into an ox; 12 days afterwards trypanosomes appeared in the blood of this ox.

Experiment 1870.

Remarks.—Fifty laboratory-bred flies were fed on a calf whose blood contained *Trypanosoma vivax*; 10 and 12 days afterwards all the flies were killed and dissected. No flagellates were found in any part of the flies.

Table 11.—Number of Flies found Infected with Trypanosomes in the Experiments with Laboratory-Bred Flies and *Trypanosoma vivax*.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
1591	50	+	3	Not injected.	Probosces infected.
1698	68	+	5	"	" "
1700	60	+	22	+	" "

In these experiments 178 flies were used, and of these 30, or 17 per cent., became infected. A curious fact is that in all the

30 flies, with the exception of one, the infection was confined to the proboscis. There was a feeling in the minds of the members of the Commission that this growth of flagellates in the proboscis was something quite characteristic of *Trypanosoma vivax*. Only on one occasion was this development of trypanosomes in the proboscis seen after feeding laboratory-bred flies on blood which was known to contain nothing but *Trypanosoma gambiense*.

(g.)—*The Development of Trypanosoma nanum in Lake-shore Glossina palpalis.*

Table 12.—The Development of *Trypanosoma nanum* in Lake-shore *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1035	120	3	3	3

Only one experiment was carried out with *Trypanosoma nanum* and Lake-shore *Glossina palpalis*. It is unsatisfactory, as trypanosomes appeared in the first healthy goat a few days after the fly had fed on the infected animal. None of the flies which were dissected showed any flagellates in their alimentary canal.

Experiment 1035.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. June 21—23	1—2	Flies fed on infected goat.	+		120 flies used.
„ 24—25	3—4	Flies starved.			
„ 26—	5—10	Flies fed on healthy goat, 1171.			
July 1		Flies starved.			
July 2—4	11—13	Flies fed on healthy goats, 1257 and 1258.	—		Aug. 3, goat 1257 died; negative.
„ 5—	14—42	Flies fed on healthy goats, 1257 and 1258.			Aug. 25, 9 remaining flies dissected; negative.
Aug. 2					
Aug. 3—24	43—64	Flies fed on goat 1258.			

Remarks.—One hundred and twenty wild flies were fed for three days on an infected goat. Five days after their first feed they infected a healthy goat, or, at least, trypanosomes resembling *Trypanosoma nanum* appeared in the blood. They failed to infect other healthy goats, although they were fed up to the 64th day after their first infected feed. Nine remaining flies dissected on the 65th day were negative for flagellates.

(h.)—*The Development of Trypanosoma nanum in Laboratory-Bred Glossina palpalis.*Table 13.—The Development of *Trypanosoma nanum* in Laboratory-Bred *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1738	100	3	—	—

Only one experiment was carried out with laboratory-bred flies. The result was negative.

Experiment 1738.

Remarks.—One hundred laboratory-bred flies were fed for three days on an ox whose blood contained *Trypanosoma nanum*, and then on a healthy goat for 40 days. This goat remained healthy, and all the flies when dissected were found free from flagellates.

(i.)—*The Development of Trypanosoma brucei in Laboratory-Bred Glossina palpalis.*Table 14.—The Development of *Trypanosoma brucei* in Laboratory-Bred *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1628	50	3	—	—
1925	150	4	—	—
2201	60	6	—	—

All three experiments were negative. The flies fed on healthy animals for 60, 50, and 50 days respectively. Two hundred and four flies were dissected, with negative results.

[Two experiments, to ascertain if *Trypanosoma brucei* can be transmitted mechanically by *Glossina palpalis*, with an interval of 10 and 20 minutes between feeding the flies on infected and healthy animals, gave negative results.]

(j.)—*The Development of Trypanosoma uniforme in Laboratory-Bred Glossina palpalis.*

Two experiments were carried out, to ascertain if *Trypanosoma uniforme* could be transmitted from sick to healthy oxen. Unfortunately, the healthy oxen accidentally contracted an infection

with *Trypanosoma pecorum* before the experiment was completed. On discovering this accidental infection the transmission experiments were at once stopped and all the flies killed off and dissected, to see if any development of *Trypanosoma uniforme* had taken place in their probosces or intestines.

In the first experiment (1709) 38 flies were dissected between the 23rd and 27th day after feeding upon an ox infected with *Trypanosoma uniforme*. The probosces of these flies were carefully examined in the fresh preparation. None were found to be infected with flagellates. Stained preparations of the salivary glands and intestines were subsequently examined, with negative results, with the exception of two flies, in the gut of which a few *Trypanosoma pecorum* were found amongst the blood recently imbibed from the accidentally infected ox.

In the second experiment (1710) 28 flies were dissected between the 20th and the 48th day after feeding on a *Trypanosoma uniforme*-infected ox. The probosces and intestines of all these flies were carefully examined, with negative results in each case.

Although the primary purpose of these experiments was frustrated by the accidental infection of the healthy oxen in Nature, the record of the fly dissections is here quoted in support of the idea that *Trypanosoma uniforme* is a species quite distinct from *Trypanosoma vivax* or *Trypanosoma gambiense*. The Commission find that whenever laboratory-bred flies were fed upon oxen or goats infected with *Trypanosoma vivax*, a large proportion of flies subsequently developed a growth of flagellates within their probosces, and flies fed upon oxen infected with *Trypanosoma gambiense* developed a growth of trypanosomes within their intestines. In 66 flies dissected after having imbibed *Trypanosoma uniforme*-infected blood no growth of flagellates was detected within them.

Conclusions.

1. That *Trypanosoma gambiense* multiplies in the gut of about one in every 20 *Glossina palpalis* which have fed on an infected animal.
2. That the flies become infective, on an average, 34 days after their first feed.
3. That a fly may remain infective for 75 days.
4. That *Trypanosoma pecorum*, *Trypanosoma vivax*, and *Trypanosoma nanum* may also multiply in *Glossina palpalis*, which must therefore be looked upon as a possible carrier in these diseases.
5. That multiplication in the tube of the proboscis is characteristic of *Trypanosoma vivax*.
6. That the experiments with *Trypanosoma brucei* and *Trypanosoma uniforme* and *Glossina palpalis* gave negative results, but the experiments were too few in number to be conclusive.

3.—FURTHER RESEARCHES ON THE DEVELOPMENT OF *TRYPANOSOMA GAMBIESE* IN *GLOSSINA PALPALIS*.*

The object of these experiments was to try to discover if there is any definite cycle of development of the trypanosome of Sleeping Sickness in the tsetse fly, *Glossina palpalis*, and if the late or renewed infectivity of the fly coincides with any phase in this development.

The mode of experimentation was to feed a cageful of *laboratory-bred* tsetse flies on an animal whose blood contained numerous trypanosomes, and at the end of various times to kill the flies and examine their intestinal contents. This was done for periods of one day, two days, three days, and so on, up to 56 days. The microscopical examination of preparations made from the intestinal contents on the various days gave information as to the number and appearance of the trypanosomes.

After the infective feed or feeds the flies were fed every day on a healthy animal, so that by the appearance of trypanosomes in the animal's blood the day on which one or more of the flies became infective could be arrived at.

Method Used in the Examination of the Flies.

The flies were killed by exposing them to the vapour of chloroform. After being killed the proboscis and pharynx were removed and examined under a cover-glass with the high and low powers. The terminal segment of the abdomen of the fly was then snipped off, and the whole abdominal viscera gently pressed out. This was moistened with a little normal saline solution, and the gut unravelled without rupturing. The proventriculus and crop were often pulled out intact with the gut. The whole thoracic and abdominal tract could then be laid out in line and examined under a low power.

In taking out the gut it was generally possible to draw out with it the abdominal portion of the salivary glands, which could then be separated without contamination from accidental rupture of the gut. If the salivary glands or proventriculus remained behind they were dissected out after removal of the gut. In every case these organs were thoroughly washed in three changes of normal saline solution, in order to minimise the chance of their being contaminated by accidental rupture of the intestines.

The stained specimens were examined day after day, and coloured drawings, at a magnification of 2,000 diameters, made of all the different forms met with. The drawings of the trypanosomes found in the proboscis, proventriculus, fore-gut, mid-gut, hind-gut, proctodæum, and salivary glands, were kept separate, so that a series of drawings of trypanosomes taken from any one

* Reprinted from the 'Proceedings' of the Royal Society, B, Vol. 83, 1911.

part, from the first day of infection to the 56th day, could be compared.

By arranging these drawings on a wall the horizontal layers would represent the contents of, say, the fore-gut from one day onwards, while the vertical rows would represent the trypanosomes found in the whole length of a fly for one day. More than six hundred drawings were made in this way, so that it seems impossible that any important form can have been left out.

General Considerations regarding the Development of the Trypanosomes in the Fly.

Let us now take a general view of the types of trypanosomes found in the various parts. It is evident that very few of the six hundred drawings can be reproduced; a few types, taken here and there, must suffice. For the first three or four days trypanosomes are found in all the flies, but at the end of six or seven the trypanosomes have disappeared out of many of them. That is to say, it is only in a certain percentage that further development takes place. In one series this was 8 per cent. In 92 per cent., then, of flies which imbibe infected blood, the trypanosomes simply degenerate and die out within the first few days. In 8 per cent., on the other hand, the trypanosomes find conditions more favourable for development, and increase to a marvellous extent, filling the whole of the fore-gut, mid-gut, and hind-gut with countless swarms of trypanosomes.

How long this development continues is unknown. It is considered probable that it continues for the remainder of the fly's life, and this has been proved by experiment up to 96 days.

What the conditions in the intestine of the fly are, which render this development possible, are also unknown. It was thought that it might depend on the reaction of the intestinal fluids. That is, however, found on examination to be faintly acid in all flies, whether development has taken place or not. The presence of bacteria also seems to have no connection with the phenomenon. Sex, moreover, has no influence; development occurs in as many males as females.

Types of Trypanosoma gambiense found in the Alimentary Canal.

It would serve no good purpose to describe separately, day by day, the types found in the various parts of the alimentary canal, as they run into each other in such a way that any classification of them seems impossible.

The following table represents, approximately, the numbers found in the different parts of the alimentary canal at various times after infection. The — sign means that an examination was made and nothing found. ± means few. +, many. ++, very many. +++ , swarming. If no sign, then no examination has been made.

Table 15.—Number of Trypanosomes found in the Various Parts
of the Alimentary Canal and Salivary Glands.

Time, days.	Pro- boscis.	Proventri- culus.	Crop.	General smear of gut.	Fore- gut.	Mid- gut.	Hind- gut.	Procto- dæum.	Salivary glands.
1	—	—				++			—
1	—	—				+	+		—
2	—				+	+	++		—
3	—					++	++		
4	—				+	+	+	—	—
5	—	—	—		—	—	—		—
6	—	—	—	+	+	+			—
7	—	—	—	+					—
8	—	+			+	++	++		—
8	—				±	±	±		
9	—	—			±	++		—	—
9	—				+	+	+		
10	—				+	++	++		
10	—					+			
11	—	±	—	++	—	±	±		—
11	—	±	—	++	++	±			—
11	—	++			++	++	++		—
14	—	+++	—		++	++	++	—	—
14	—	++	—		++	++	++		—
15	—	—			+	+			—
17	—	—	—		±	++	++		—
17	—	++		—	++	++			—
18	—	—			++	+	++		—
18	—					++	++		—
20	—	++			++	++	++	±	—
21	—			+++					
22	—			++					
23	—				+	+	+		
24	—				+++	+++	+++		
25	—	—	—		+	+++	++		+
28	—			++		++			+
30	—	+			++	++			+
30	—	+			++	++	±		—
31	—	+	+		+++	++			—
31	—			+					
34	—			++	++	+++	+++	—	—
35	—	—			+	++	++		—
36	—			+++	+++	+++	+		+
36	—	—		+++	++	+++			—
36	—	++		+++					
37	—			++					
40	—			++					
40	—			++					
42	—	++		+	++				+
43	—			++					++
44	—	+			++	+++			+
44	—				+	++	++		+
44	—	+			+	+	+		+
46	—	+			+++	+++	+++		++
49	—	—	—		+	±			—
51	—	—	—		+	+	++		—
53	—	—			±	±			—
53	—	—			+	++			+
53	—		—			±			—
56	—			++					+

The Proboscis.—In our experience *Trypanosoma gambiense* is never found in the proboscis of *Glossina palpalis*, except immediately after an infected feed, when for a short time blood containing trypanosomes may be seen in the lumen of the proboscis. This is very different from what obtains in an infection by *Trypanosoma vivax*, in which case the proboscis is alone infected.

Proventriculus.—As seen from the above table, this part of the alimentary canal is sometimes found empty when the remainder of the gut is swarming.

Fore-, Mid-, and Hind-gut.—It is here that the greatest development of the trypanosomes is found. Among the extraordinary numbers and diversity of type it is difficult or impossible to find one's way. Generally speaking, the trypanosomes found during the first few days are merely degenerating blood forms. After this there appears a type of trypanosome which remains dominant throughout the whole developmental period. This is a long, moderately broad form, the protoplasm staining well, without granules or vacuoles, having an oval compact nucleus situated in the centre of the body, a small round micronucleus lying at some distance from the elongated snout-like posterior extremity, the undulating membrane narrow and simple, and the flagellum proceeding little, if anything, beyond the protoplasm of the cell. The flagellum also appears very frequently to arise from a pink-coloured body situated near the micronucleus, an appearance never seen in the normal blood trypanosomes.

This seems to be the healthy normal developing type in the intestine of the fly. It is seen in all parts of the intestine and at all times. It forms masses of innumerable individuals alike in size and shape. When a fresh supply of blood is taken in by the fly this type can be imagined to multiply with extraordinary rapidity. When the blood supply runs low then this type can also be imagined as degenerating and disappearing just as rapidly. The host of diverse forms which thus arises beggars description. Some are round or oval in shape, 3 or 4 microns in diameter, with or without a flagellum. From this simple form all shapes and sizes can be seen up to the huge shapeless mass of protoplasm, multi-nucleated and multi-flagellated.

In our experience, the long narrow forms, described by some observers as "male" forms, are exceedingly rare, and it seems impossible to ascribe to them any very important rôle in the process of development. Crithidia-like forms are also exceedingly rare—that is, forms with the micronucleus close to or anterior to the nucleus.

Types of Trypanosoma gambiense found in the Salivary Glands.—On referring to Table 15 it will be seen that trypanosomes did not appear in these glands until the twenty-fifth day, but that after this time they were usually present. In our opinion, this invasion of the salivary glands is of the greatest importance in the history of the development of *Trypanosoma gambiense* in *Glossina palpalis*. Here it may be parenthetically remarked that, because one kind of development takes place with one species of trypanosome and one species of tsetse fly, it by no means follows that the same thing will occur either with another species of

trypanosome or another species of fly. Each combination must be worked out separately and nothing left to analogy. *Trypanosoma vivax* and *Glossina palpalis* afford a striking example of this.

In the development of *Trypanosoma gambiense* in *Glossina palpalis* one circumstance, which we think of prime importance, emerges, and that is, that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal blood-type. It must not be imagined, however, that the salivary glands show no other forms but this blood-type. On the contrary, there are many other forms seen; but here only are found trypanosomes apparently identical with the short and stumpy forms found in the blood. What causes or leads up to this reversion to the blood-type in the salivary glands is quite unknown, but, as will be seen later, the *Glossina palpalis* does not become infective by biting until this invasion of the salivary glands takes place.

How the trypanosomes find their way into the salivary glands is also quite unknown. It seems highly improbable that they pass from the alimentary canal by way of the salivary duct, and as they are never found in the body-cavity, it is also difficult to see how they can make their way directly from the intestine to the abdominal portion of the salivary glands.

Illustrations of Various Modifications in Shape of Trypanosoma gambiense in Glossina palpalis.



× 2000.

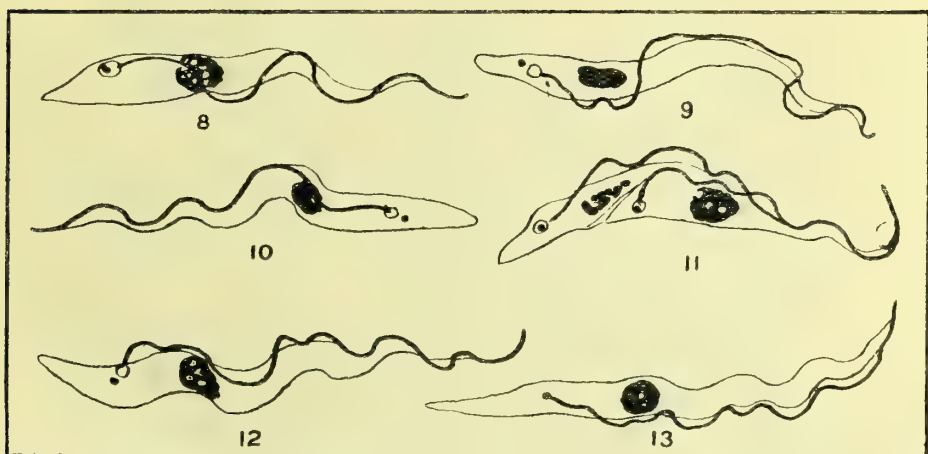
FIGS. 1-3.—Normal blood parasites (*Trypanosoma gambiense*).

FIG. 4.—24 hours after ingestion by the fly.

FIGS. 5 and 6.—48 hours after ingestion by the fly.

FIG. 7.—96 hours after ingestion by the fly.

Figs. 1-7 represent the trypanosomes as they appear in the intestine of *Glossina palpalis* during the first few days. Figs. 1-3 are ordinary blood forms, as seen immediately after the fly has fed, and before any change has taken place. Figs. 4-7 represent the process of degeneration which takes place during the first four days. The body swells up, the nucleus breaks up, and the cytoplasm becomes vacuolated.



× 2000.

FIG. 8.—*Trypanosoma gambiense* from fore-gut, 8 days after infected feed.

FIG. 9.—*T. gambiense* from proventriculus, 14 days after infected feed.

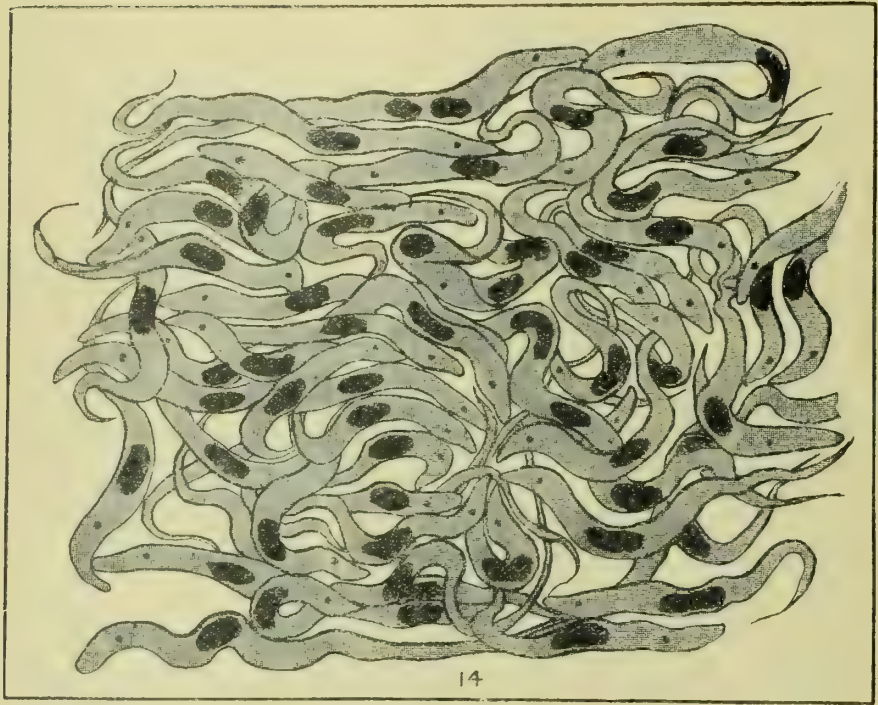
FIG. 10.—*T. gambiense* from fore-gut, 18 days after infected feed.

FIG. 11.—*T. gambiense* from mid-gut, 25 days after infected feed.

FIG. 12.—*T. gambiense* from mid-gut, 34 days after infected feed.

FIG. 13.—*T. gambiense* from fore-gut, 44 days after infected feed.

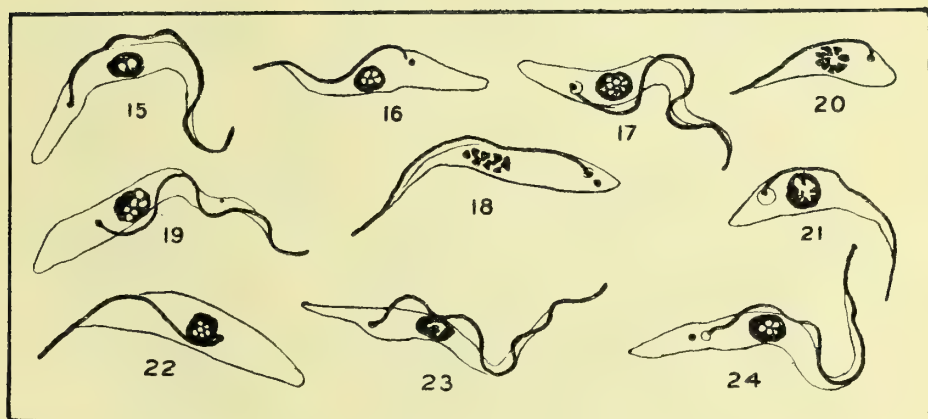
Figs. 8-13 represent what we consider to be the normal reproductive or developing type found throughout the intestine during the whole period of development. It is to be noted that this form is longer and broader than the normal broad form; the protoplasm is clear, and stains readily and evenly, and this cell looks normal. The nucleus is compact and situated nearer the posterior extremity than the anterior. The micronucleus is small and round, lying at some distance from the elongated posterior extremity. Many dividing forms of this type can be seen. In our opinion, this is the common multiplying form, and from it arises an infinite variety of degenerating forms.



× 2000.

FIG. 14.—Part of a mass of *Trypanosoma gambiense* from the mid-gut.

This figure is given in order to try to convey some idea of the enormous proliferation of trypanosomes which takes place in the intestine of *Glossina palpalis*. Throughout the whole length of the alimentary canal, from the proventriculus to the proctodæum, this condition is frequently seen, and in the living condition presents an extraordinary spectacle. Looked at through the wall of the intestine the matted masses are seen in active movement, swaying about and wriggling in every direction. They do not appear to be attached to the wall of the intestine, but move about freely, and when the intestinal wall is burst pour out in countless numbers. The trypanosomes evidently belong to the type figured in the preceding sketch, called the normal reproductive type.



× 2000.

FIG. 15.—*Trypanosoma gambiense* from hind-gut, 8 days after infected feed.

FIGS. 16, 17, and 18.—*T. gambiense* from mid-gut, 14, 20, and 30 days after infected feed.

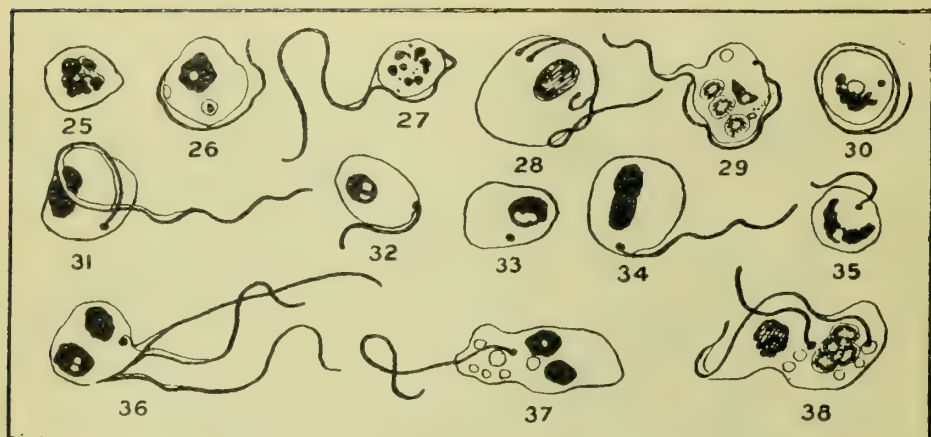
FIG 19.—*T. gambiense* from hind-gut, 34 days after infected feed.

FIGS. 20 and 21.—*T. gambiense* from proventriculus, 36 days after infected feed.

FIG. 22.—*T. gambiense* from hind-gut, 44 days after infected feed.

FIGS. 23 and 24.—*T. gambiense* from mid-gut, 46 and 53 days after infected feed.

Figs. 15-24 represent smaller forms which are fairly common and occur throughout the intestine and at all times. The examples given above are taken from the eighth day to the fifty-third day. They are called by us "small developmental forms," since they resemble the larger in having clear protoplasm and a compact nucleus. Dividing forms are often seen.



× 2000.

FIG. 25.—*Trypanosoma gambiense* from mid-gut, 8 days after infected feed.

FIG. 26.—*T. gambiense* from fore-gut, 11 days after infected feed.

FIGS. 27 and 28.—*T. gambiense* from mid-gut, 16 and 17 days after infected feed.

FIGS. 29, 30, and 31.—*T. gambiense* from fore-gut, 17 and 18 days after infected feed.

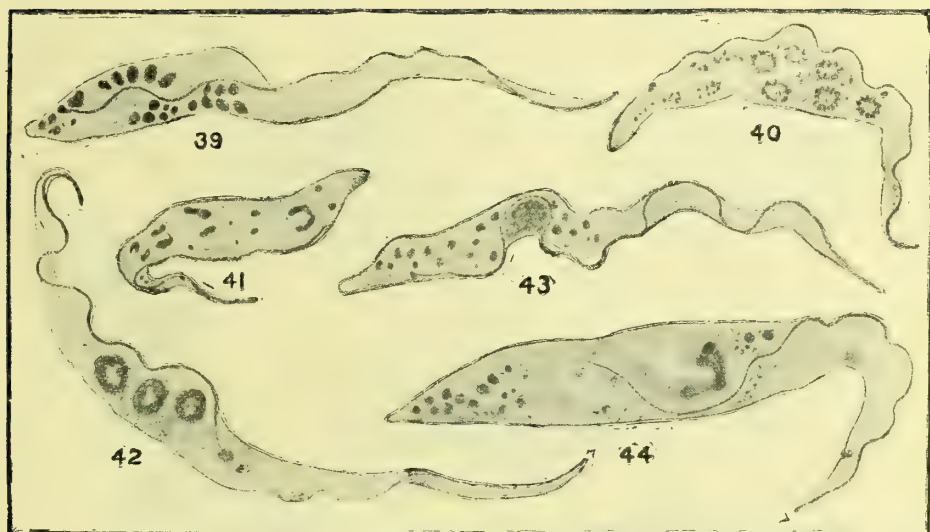
FIG. 32.—*T. gambiense* from hind-gut, 20 days after infected feed.

FIGS. 33 and 34.—*T. gambiense* from fore-gut, 24 days after infected feed.

FIGS. 35, 36, and 37.—*T. gambiense* from mid-gut, 24, 44, and 46 days after infected feed.

FIG. 38.—*T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 25-38 represent round and irregularly-shaped forms of the parasite, taken from the eighth to the forty-sixth day of development. It is impossible to say what is exactly the origin of these forms—whether by the segmentation of large masses, or simply by the division and subdivision of irregular forms. Some of the examples figured are evidently dividing, as they show several nuclei and flagella. Whether the round aflagellar forms correspond to the so-called “latent” forms of various writers it is impossible to say. Those possessing flagella were active during life. In our opinion, they may be looked upon as part of the degenerative processes which are constantly taking place in the intestine of the fly.



× 2000.

FIG. 39.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.

FIG. 40.—*T. gambiense* from fore-gut, 17 days after infected feed.

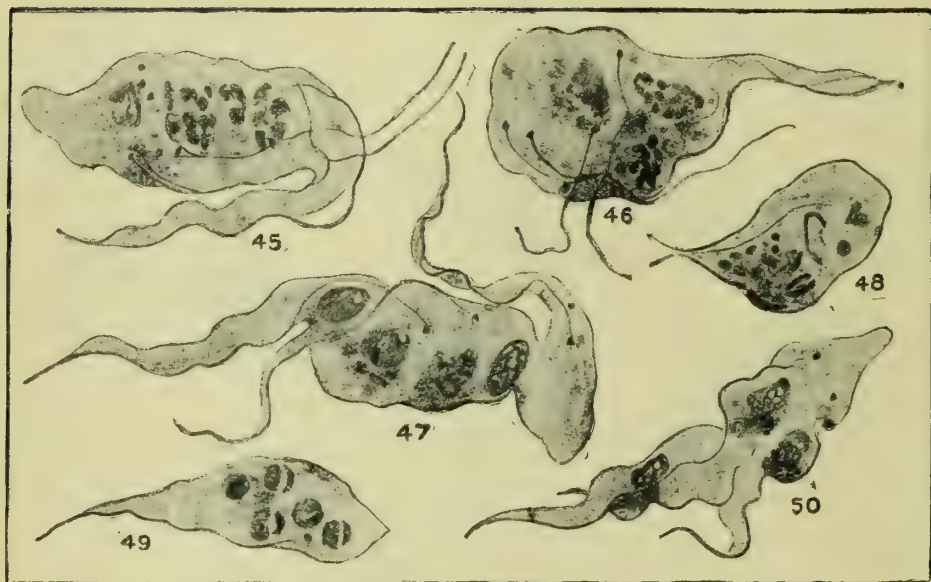
FIG. 41.—*T. gambiense* from fore-gut, 17 days after infected feed.

FIG. 42.—*T. gambiense* from hind-gut, 34 days after infected feed.

FIG. 43.—*T. gambiense* from mid-gut, 34 days after infected feed.

FIG. 44.—*T. gambiense* from hind-gut, 46 days after infected feed.

Figs. 39-44 represent what appear to us to be degenerative forms of the "normal reproductive type." They are of all sizes and shapes, and the cell-contents are scattered over with broken-up nuclei, or at least granules of some stainable substance. Figs. 39, 43, and 44 are also vacuolated.



× 2000.

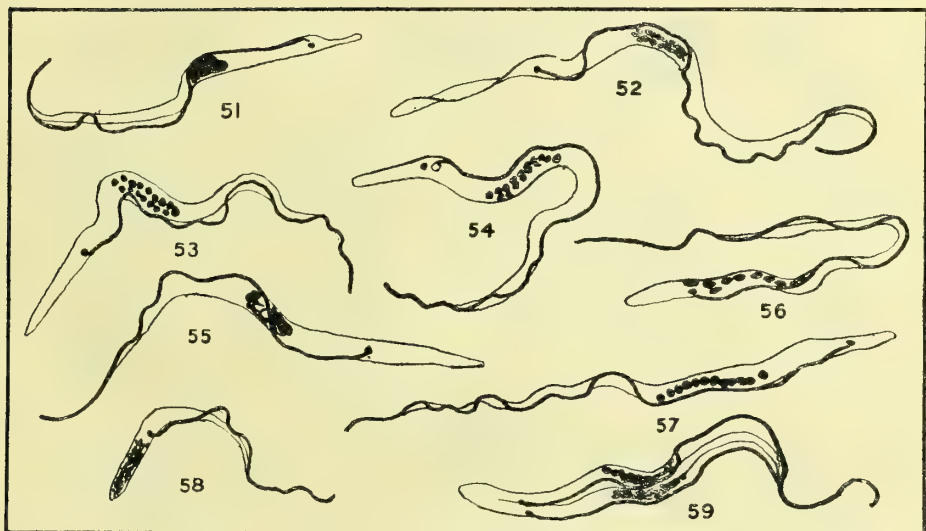
FIGS. 45, 46, and 47.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.

FIG. 48.—*T. gambiense* from fore-gut, 17 days after infected feed.

FIG. 49.—*T. gambiense* from proventriculus, 30 days after infected feed.

FIG. 50.—*T. gambiense* from mid-gut, 46 days after infected feed.

Figs. 45-50 represent some of the more exaggerated types of degenerative forms. As will be seen from the drawings, they are huge, mis-shaped masses of protoplasm, multi-nucleated and, as a rule, multi-flagellated.



× 2000.

FIG. 51.—Slender type of *Trypanosoma gambiense* from proventriculus, 17 days after infected feed.

FIG. 52.—Slender type of *T. gambiense* from mid-gut, 17 days after infected feed.

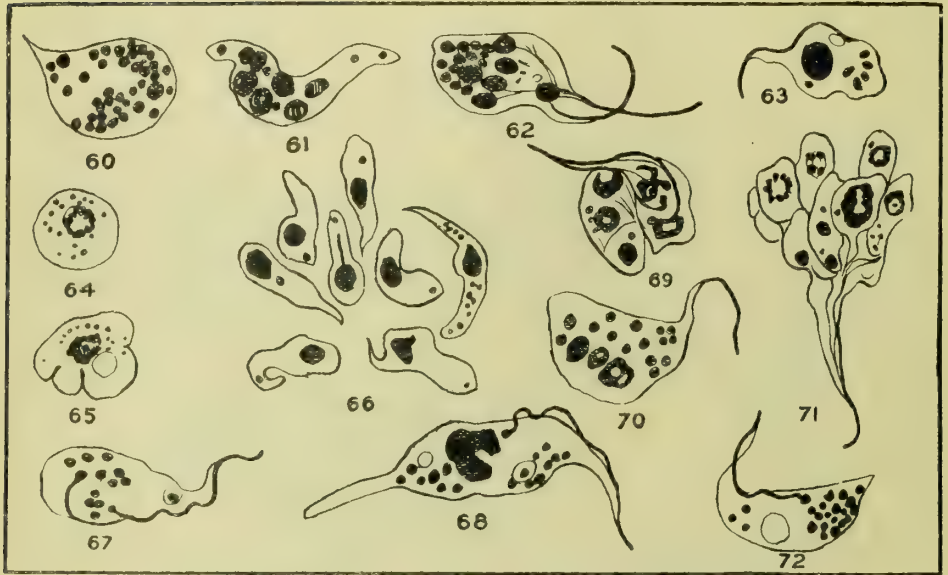
FIG. 53.—Slender type of *T. gambiense* from hind-gut, 20 days after infected feed.

FIGS. 54 and 55.—Slender types of *T. gambiense* from fore-gut, 24 and 30 days after infected feed.

FIGS. 56 and 57.—Slender types of *T. gambiense* from proventriculus, 44 days after infected feed.

FIGS. 58 and 59.—Slender types of *T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 51-59 represent various varieties of the slender type of *Trypanosoma gambiense* found in the intestine of *Glossina palpalis*. Fig. 59 is dividing. Figs. 58 is a Crithidia type. These slender and crithidial types are uncommon, and no special connection between them and the onset of infectivity in the fly has been made out.



× 2000.

FIGS. 60 and 61.—*Trypanosoma gambiense* from salivary glands, 25 days after infected feed.

FIG. 62.—*T. gambiense* from salivary glands, 30 days after infected feed.

FIG. 63.—*T. gambiense* from salivary glands, 42 days after infected feed.

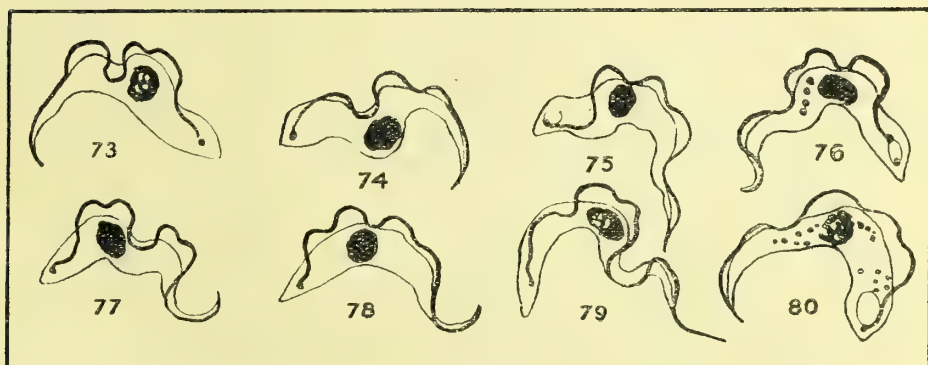
FIGS. 64, 65, and 66.—*T. gambiense* from salivary glands, 44 days after infected feed.

FIGS. 67 and 68.—*T. gambiense* from salivary glands, 46 days after infected feed.

FIGS. 69 and 70.—*T. gambiense* from salivary glands, 53 days after infected feed.

FIGS. 71 and 72.—*T. gambiense* from salivary glands, 56 days after infected feed.

Figs 60-72 represent aberrant forms of *Trypanosoma gambiense* seen in the salivary glands. The first thing to be noted is the fact that the trypanosomes do not appear in the salivary glands until a late date; in this case the earliest appearance is 25 days after the infected feed. How they reach these glands is up to the present unknown. In what shape they reach the glands is also unknown. Figs. 64, 65, 69 and 71 might be said to point to some process of segmentation, of which the finished product is represented by fig. 66.



× 2000.

FIG. 73.—*Trypanosoma gambiense* from salivary glands, 34 days after infected feed.

FIG. 74.—*T. gambiense* from salivary glands, 42 days after infected feed.

FIG. 75.—*T. gambiense* from salivary glands, 43 days after infected feed.

FIGS. 76, 77, and 78.—*T. gambiense* from salivary glands, 46 days after infected feed.

FIGS. 79 and 80.—*T. gambiense* from salivary glands, 56 days after infected feed.

When alluding, generally, in a previous part of this paper to the types of *Trypanosoma gambiense* found in the salivary glands, it was said that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal type found in the blood. Figs. 73-80 illustrate this reversion. By comparing them with figs. 1, 2, and 3, which represent normal blood trypanosomes, it will be seen that they are very similar to the short and stumpy form found in the blood. No such forms have ever been seen in any other part of the fly, and we would suggest that the occurrence of these forms in the salivary glands, coinciding as it does with the renewed infectivity of the fly, is more than a mere coincidence.

Infectivity of Trypanosoma gambiense after its Development in Glossina palpalis, as shown by the Bites of the Flies giving rise to the Disease in Healthy Animals.

As has been shown in a previous paper,* the fly ceases to be infective by biting within a short time of its infective feed. From this time on for some 28 days the fly remains non-infective.

The following table illustrates this:—

Table 16.—The Result of the Bites of Flies at Varying Periods after an Infective Feed.

No. of days after infective feed.	Result of bites.	No. of days after infective feed.	Result of bites.
1	—	28	+
2	—	30	—
3	—	31	—
4	—	34	+
5	—	35	—
6	—	36	—
8	—	37	+
9	—	40	+
11	—	40	+
14	—	42	+
15	—	43	+
17	—	44	+
18	—	46	+
20	—	51	—
23	—	53	+
25	—	56	+

From this table it will be seen that in this series of experiments the flies first became infective 28 days after the infective feed, and that after this time the flies are usually found to be capable of giving rise to the disease by their bites.

* 'Proceedings' of the Royal Society, B, vol. 82, 1910, p. 498, and p. 66 of this Report.

It was stated above that the most important discovery made in this research is the connection between the invasion of the salivary glands and the infectivity of the fly. That this appears to be so is shown in the following table:—

Table 17.—To show Correlation between the Invasion of the Salivary Glands of *Glossina palpalis* by *Trypanosoma gambiense* and Infection by the Bite of the Fly.

Experiment No.	No. of days after infective feed.	Bites of fly infective or non-infective.	Salivary glands.	Remarks.
1910	1	—	—	
1910	2	—	—	
1910	3	—	—	
1910	4	—	—	
1910	5	—	—	
1910	6	—	—	
1910	7	—	—	
1894	8	—	—	
1894	9	—	—	
2216	10	—	—	
1894	11	—	—	
1871	14	—	—	
1693	15	—	—	
1945	17	—	—	
1945	18	—	—	
1945	20	—	—	
1718	25	—	+	Blood-type not present.
1602	28	+	+	Blood-type present.
1801	30	—	—	
1945	31	—	—	
1760	34	+	+	
1769	36	—	—	" "
1712	42	+	+	" "
2034	43	+	+	" "
1549	44	+	+	" "
2034	44	+	+	" "
1706	51	—	—	" "
1566	53	+	+	" "
1651	56	+	+	" "

From the above table it is seen that the salivary glands first become invaded 25 days after the infecting feed. In this case the monkey did not become infected. This may have been due to an accident, such as this particular fly not biting the monkey, or the invasion of the salivary glands only taking place after the fly had bitten, or to the fact that the blood-type of trypanosome was not present. With this exception all the other experiments, 12 in number, confirm the hypothesis that the fly does not become infective until the salivary glands are invaded.

Another point which comes out with striking clearness is, that in all the salivary glands from flies which gave a positive result, trypanosomes similar to the short and stumpy blood-type were invariably present.

It is to be hoped that before long the result of this work will be confirmed, and so added to, that the whole story of the development of the trypanosomes within the fly, and their passage into the salivary glands, will be unfolded.

Conclusions.

1. In the course of the development of *Trypanosoma gambiense* in *Glossina palpalis* the proboscis does not become involved, as in the case of some other species.

2. A few days after an infective feed the trypanosomes disappear out of the great majority of the flies, but in a small percentage this initial disappearance is followed by a renewed development.

3. After a very short time the flies which have fed on an infected animal become incapable of conveying the infection by their bites, and this non-infectivity lasts for some 28 days, when a renewed or late infectivity takes place.

4. A fly in which this renewed or late infectivity occurs can remain infective for at least 96 days.

5. An invasion of the salivary glands occurs at the same time as this renewal of infectivity, and without this invasion of the salivary glands there can be no infectivity.

6. The type of trypanosome found in the salivary glands when the fly becomes infective is similar to the short stumpy form found in vertebrate blood, and it is believed that this reversion to the blood-type is a *sine qua non* in the infective process.

4.—FURTHER RESEARCHES ON THE DEVELOPMENT OF TRYPANOSOMA VIVAX IN LABORATORY-BRED GLOSSINA PALPALIS.

In a previous paper* it was shown that *Trypanosoma vivax* readily develops in *Glossina palpalis*, and that this tsetse fly can convey the parasite from an infected to a healthy animal after an interval of 20 to 30 days. It was further shown that the infectivity of the fly was associated with a growth of flagellates within the proboscis, and the proboscis alone, of some 17 per cent. of flies which had imbibed infected blood.

During the early part of 1910 transmission experiments with laboratory-bred *Glossina palpalis* were repeated, with the object of ascertaining the nature of the development of this trypanosome within the fly. It was impossible, however, to do more than two experiments.

The flies were first fed upon a goat infected with *Trypanosoma vivax*, and, subsequently, upon a healthy calf or goat until one

* 'Proceedings' of the Royal Society, B, Vol. 82, 1910, p. 381, and p. 26 of this Report.

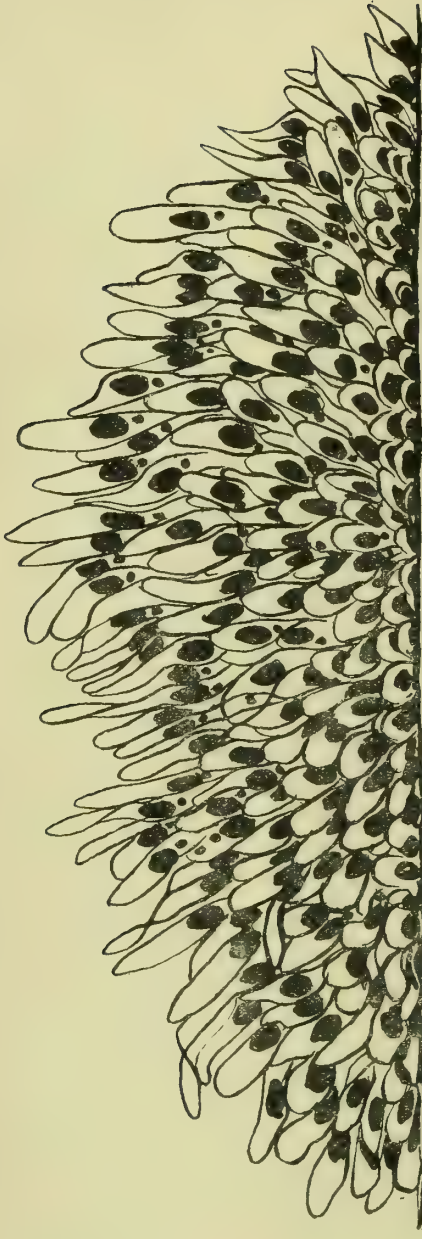


FIG. 81. *Trypanosoma vivax* in proboscis of *Glossina palpalis*.

× 2000.

of these showed *Trypanosoma vivax* in its blood. At various intervals during the experiments flies were taken from the cages and dissected, and in most cases whenever the proboscis was found to be infected it was injected, subcutaneously, into a healthy goat. The following table gives the main results of the first experiment:—

Table 18.

Experiment.	No. of flies used.	<i>T. vivax</i> transmitted from goat to healthy calf.	No. of days before flies became infective.	No. of infected flies found.	Results of injection of infected flies.
		Result.			
2183	100	Positive.	17	27	Negative.

Remarks.—In estimating the number of days after the infected feed which must elapse before the flies become infective, 7 days has been allowed as the minimum period required for the incubation of this trypanosome in the blood of a vertebrate host. Eleven flies were dissected at various intervals between the 8th and the 17th day after imbibing infected blood; none of these contained flagellates.

On the 19th day, *i.e.*, 2 days after the cage of flies became infective, 5 flies were taken out and dissected. One of these was infected with Crithidia-like flagellates, which were attached in clusters to the inner surface of the labrum of the proboscis (Fig. 81). They were fixed to the wall of the proboscis by their flagella. Single parasites were swimming rapidly about within the tube of the proboscis. No flagellates were found in the intestine or in any other part of the fly.

Four flies were dissected between the 20th and 24th day; none of these contained flagellates.

Ten flies were dissected on the 26th day after their infected feed. Four of these were infected with flagellates in the proboscis. All the infected probosces were injected into goat 2278, with negative results.

Eleven flies were dissected on the 27th day after their infected feed. Three of these contained flagellates in the proboscis; none were found in the crop, proventriculus, or any part of the gut. The infected probosces were injected, subcutaneously, into a goat; but the goat did not become infected. Examination of stained preparations of the salivary glands, proventriculus, crop, and intestines was negative.

Fifteen flies were dissected 30 days after imbibing infected blood. Eight of them had Crithidia-like parasites within the proboscis. The intestine was not infected. Salivary glands not examined; no stained preparations were made.

The remaining 20 flies of this experiment were dissected 33 days after their infected feed. The probosces and alimentary

tracts of all the flies were negative for flagellates. Stained preparations were made of the salivary glands, proventriculus, thoracic intestine, crop, and gut; on examination of these preparations no flagellates were detected.

The results of the other experiment are shown in the following table:—

Table 19.

Experiment.	No. of flies used.	<i>T. vivax</i> transmitted from goat to healthy goat.	No. of days before flies became infective.	No. of infected flies found.	Results of injection of infected flies.
		Result.			
2184	100	Positive.	28	5	Negative.

Remarks.—It will be seen that this batch of flies did not become infective until 28 days after their feed on infected blood.

Thirty-five flies were dissected between the 8th and the 26th day; none of these flies were infected with flagellates.

Eight of the remaining flies were dissected 35 days after they had imbibed infected blood. Of these 3 males and 2 females showed heavy infection of the proboscis with flagellates resembling those described in Experiment 2183. The pharynx also was infected in each case. The intestines though carefully examined were negative for flagellates. The probosces of these flies were not inoculated into any animal.

In another experiment (1870) 50 laboratory-bred flies were fed upon a calf showing *Trypanosoma vivax* in its blood. Ten days after the infected feed all the flies were dissected and carefully examined for flagellates, with negative results.

In a similar experiment (1831) performed with 40 laboratory-bred flies infected with *Trypanosoma vivax* from a goat, the flies were all killed and dissected on the 15th day after their infected feed. Three of these flies contained a few Crithidia-like parasites within the proboscis. Figs. 1, 2, 3, and 4, of Plate 1, represent these parasites. The intestines of the three infected flies and of all the others were smeared, fixed, stained, and carefully examined. The salivary glands of the infected flies were also examined. No sort of flagellated parasite was found in any of these preparations. Unfortunately, the healthy goat, on which these infected flies were fed, was accidentally killed before the incubation period of the disease had elapsed. (See Plate 1, p. 54.)

In two experiments carried out with the object of ascertaining if birds could become infected with *Trypanosoma vivax*, laboratory-bred flies were fed first upon an infected goat and then for 40 days upon a fowl. The fowls proved to be insusceptible to the infection, although 8 per cent. of the flies so fed developed

an infection of Crithidia-like parasites within the proboscis. In no other part of the flies' anatomy were flagellates found.

In connection with experiments to ascertain the fate of *Trypanosoma vivax* within *Glossina palpalis*, a total of 256 laboratory-bred flies, which had fed upon animals suffering from disease caused by this trypanosome, were dissected between the 15th and the 58th day after having imbibed infected blood. It was found that 54 of these flies, i.e., 21 per cent., harboured Crithidia-like parasites within the proboscis and pharynx.

In five experiments in which *Trypanosoma vivax* was transmitted from sick to healthy animals by laboratory-bred flies, the infective flies were dissected, and Crithidia-like parasites were invariably found in the proboscis and pharynx. The various forms found in the proboscis are figured on Plate 1. These Crithidia-like parasites vary from 16 to 35 microns in length.

Trypanosoma vivax was so rare in the blood of its vertebrate host that it was not possible to follow the destiny of the few parasites taken up in the drop of blood imbibed by a tsetse fly. The trypanosomes were never seen in the gut after 24 hours, and flagellates were never observed in the proboscis earlier than 15 days after the infected feed or later than 58 days after the fly had imbibed infected blood. In only one instance was the subcutaneous injection of infected probosces followed by the appearance of *Trypanosoma vivax* in the blood.* This experiment was several times repeated but with negative results.

The following control experiments may be cited in support of the idea that the Crithidia-like parasites found in the proboscis are developmental forms of *Trypanosoma vivax*.

Experiment 1875.—100 laboratory-bred flies—*Glossina palpalis*—were fed daily for 30 days upon a healthy ox. At the end of this period the flies were dissected and the proboscis and gut examined to see if they had become infected with flagellates. All were negative.

Experiment 1669.—50 laboratory-bred flies were fed upon a normal monkey for 30 days and then dissected. Careful examination of the proboscis and gut failed to reveal the presence of flagellates.

Conclusions.

1. *Trypanosoma vivax* develops in some 20 per cent. of *Glossina palpalis* which have fed upon an infected animal.

2. The infection in the fly is confined to the proboscis and pharynx, and this localised infection is characteristic of the development of *Trypanosoma vivax* in *Glossina palpalis*.

3. The forms of *Trypanosoma vivax* found in the invertebrate host resemble *Crithidia* in shape and in having the micronucleus alongside or anterior to the macronucleus.

* 'Proceedings' of the Royal Society, B, Vol. 82, 1910, and p. 30 of this Report (Experiment 1700).

DESCRIPTION OF PLATE 1.

× 2000.

Figs. 1, 2, 3, and 4 represent developing forms of *Trypanosoma vivax* as they appear in the proboscis of the fly 15 days after the fly fed upon an infected animal. Note the Crithidia-like appearance of the parasite. The micronucleus placed alongside or anterior to the macronucleus. Fig. 1 represents a dividing form.

Figs. 5, 6, 7, 8, and 9 represent *Trypanosoma vivax* as seen in the proboscis of the fly 34 days after feeding upon an infected animal. Fig. 5 is dividing. Note the position of the micronucleus: anterior to the macronucleus.

Figs. 10, 11, 12, and 13 represent developing forms of *Trypanosoma vivax* in the proboscis of the fly 38 days after imbibing infected blood.

Figs. 14, 15, 16, 17, and 18 represent *Trypanosoma vivax* in the proboscis of the fly 40 days after imbibing infected blood.

Figs. 19, 20, 21, 22, and 23 represent the parasites 53 days after imbibing infected blood.

Figs. 24, 25, 26, 27, 28, and 29 represent *Trypanosoma vivax* as seen in the proboscis of the fly 58 days after infection.

5.—EXPERIMENTS TO ASCERTAIN IF *TRYPANOSOMA GAMBIENSE*
DURING ITS DEVELOPMENT WITHIN *GLOSSINA PALPALIS* IS
INFECTIVE.*

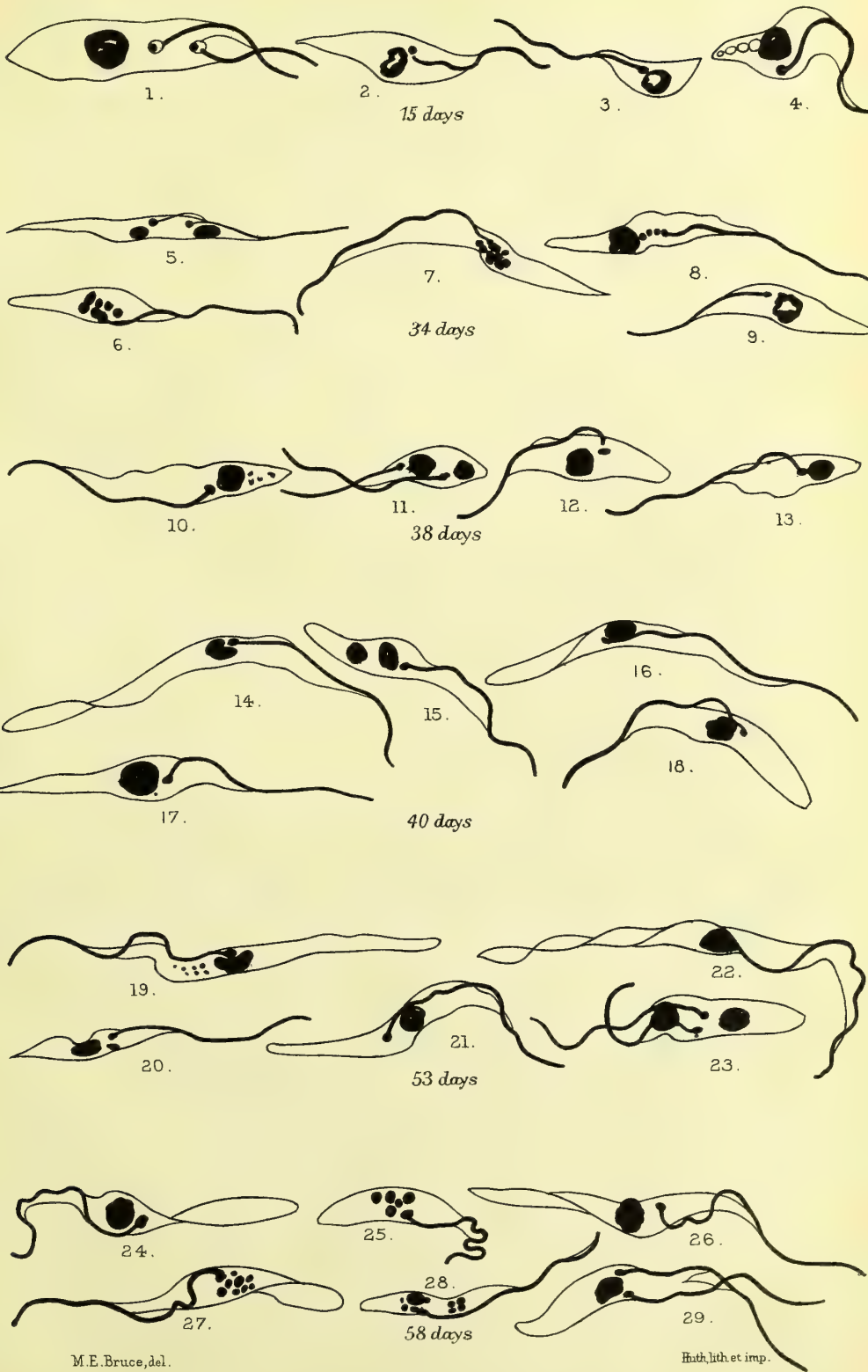
It will be remembered that the injection of chopped-up tsetse flies (*Glossina morsitans*) a short time after feeding on an infected animal, did not give rise to nagana. As this was thought to be an interesting fact, and difficult of explanation, experiments were carried out on the same lines with *Trypanosoma gambiense* and *Glossina palpalis*.

If tsetse flies (*Glossina palpalis*) are fed upon an animal whose blood contains *Trypanosoma gambiense*, the trypanosomes can be found living within the intestines of some of the flies for several days after they were ingested. In a small percentage (0·5 to 2·0 per cent.) of flies so infected, active trypanosomes may be found swarming in their intestines on any day between the seventh and the fiftieth day, or even longer, after they have been fed upon an infected animal.

A series of experiments were undertaken, to ascertain if *Trypanosoma gambiense* retained its power of causing Sleeping Sickness when inoculated, subcutaneously, into monkeys, throughout its period of multiplication within the fly, especially during the interval of some 20 days in which the bites of infected flies are harmless. In some of these experiments Lake-shore flies were used; in others, laboratory-bred flies.

The flies were fed upon a monkey whose blood contained many trypanosomes. After a pre-determined time had elapsed, the wings and legs of the infected flies were cut off, and the bodies were either chopped up and brayed in a mortar with saline solution (0·8 per cent.), or the alimentary canal alone was

* Reprinted from the 'Proceedings' of the Royal Society, B, Vol. 83, 1911.



M.E. Bruce, del.

Huth, lith et imp.

T. Vivax in Proboscis of *G. palpalis*.



removed. In many instances the gut was proved by microscopical examination to be heavily infected with trypanosomes before it was inoculated into a monkey. In flies thus found to be infected, the salivary glands also were carefully removed and washed thoroughly in several changes of normal saline solution. They were then broken up and injected, by means of a sterile syringe, into the subcutaneous tissue of the groin of a monkey.

The following table shows the result of the individual experiments as carried out with Lake-shore flies:—

Table 20.—*Glossina palpalis* and *Trypanosoma gambiense*.

Date.	Experiment No.	No. of hours after infected feed.	No. of flies injected.	Result.	Incubation period in monkey.
1909.					
Mar. 10 ...	521	$\frac{1}{2}$ hour	2	+	8 days.
Jan. 6 ...	420	$\frac{3}{4}$ "	1	—	
" 6 ...	421	1 "	2	—	
Feb. 18 ...	518	1 "	2	—	
Mar. 19 ...	604	1 "	2	+	6 "
" 19 ...	605	$1\frac{1}{2}$ hours	2	+	6 "
Feb. 18 ...	519	2 "	2	—	
June 22 ...	1038	3 "	1	+	6 "
" 22 ...	1043	3 "	1	+	6 "
Mar. 29 ...	622	4 "	8	+	7 "
June 11 ...	984	4 "	5	+	8 "
" 16 ...	1009	4 "	5	+	6 "
" 22 ...	1039	6 "	1	+	6 "
" 22 ...	1044	6 "	1	+	6 "
" 22 ...	1045	7 "	1	+	9 "
" 22 ...	1041	8 "	1	+	9 "
" 22 ...	1046	8 "	1	+	10 "
" 22 ...	1042	9 "	1	+	9 "
" 22 ...	1047	9 "	1	+	9 "
" 26 ...	1154	16 "	1	—	
" 26 ...	1155	16 "	1	—	
" 26 ...	1156	17 "	1	+	13 "
" 26 ...	1157	17 "	1	—	
" 26 ...	1158	18 "	1	+	9 "
" 26 ...	1159	18 "	1	+	13 "
Mar. 20 ...	600	19 "	5	—	
June 26 ...	1161	19 "	1	—	
Mar. 4 ...	524	23 "	6	—	
" 5 ...	585	44 "	6	—	

Remarks.—Twenty-nine experiments were carried out with Lake-shore flies and *Trypanosoma gambiense*. Eighteen of these experiments yielded a positive result, and were almost uniformly successful up to 18 hours after the infected feed. It seemed to be immaterial whether the fly was introduced whole or whether the alimentary canal alone was injected. In several instances where the gut of the fly was examined in the fresh state, swarms of flagellates natural to the fly, *i.e.* *Trypanosoma grayi*, were found, but their presence did not appear to modify the virulence of *Trypanosoma gambiense*.

The following table shows the result of the experiments as carried out with laboratory-bred flies:—

Table 21.—*Glossina palpalis* and *Trypanosoma gambiense*.

Date.	Experiment No.	No. of hours or days after infected feed.	No of flies injected.	Result.	Incubation period in monkey.
1909.					
Aug. 24 ...	1545	1 hour	1	+	7 days
" 24 ...	1546	2 hours	1	+	7 "
Feb. 18 ...	520	4 "	2	—	
Aug. 24 ...	1547	4 "	1	+	7 "
Sept. 14 ..	1681	1 day	3	—	
" 23 ...	1756	2 days	3	+	12 "
" 26 ...	1723	3 "	15	—	
Oct. 2 ...	1713	4 "	10	—	
" 3 ...	1783	5 "	11	—	
" 4 ...	1752	6 "	10	—	
*Sept. 28 ...	1721	7 "	1	—	
*Nov. 16 ...	1906	8 "	2	—	
Oct. 7 ...	1788	9 "	10	—	
*Nov. 17 ...	1909	9 "	1	—	
Oct. 8 ...	1795	10 "	8	—	
" 9 ...	1799	10 "	8	—	
*Nov. 18 ...	1911	10 "	1	—	
* " 19 ...	1913	11 "	2	—	
* " 29 ...	1923	11 "	1	—	
Oct. 11 ...	1807	13 "	5	—	
Nov. 26 ...	1864	14 "	30	—	
⊙ " 15 ...	1905	14 "	2	—	
*Oct. 3 ...	1781	15 "	1	—	
" 27 ...	1865	15 "	12	—	
" 19 ...	1838	16 "	34	—	
" 29 ...	1872	17 "	12	—	
*Dec. 13 ...	1939	17 "	1	—	
Oct. 30 ...	1873	18 "	12	—	
*Dec. 14 ...	1940	18 "	2	—	
Nov. 1 ...	1877	20 "	12	—	
* " 16 ...	1951	20 "	1	—	
Nov. 2 ...	1879	21 "	20	—	
" 4 ...	1886	23 "	15	—	
Dec. 5 ...	1887	24 "	10	—	
* " 20 ...	1958	24 "	1	+	8 "
" 6 ...	1889	25 "	22	—	
⊙Sept. 28 ...	1749	28 "	1	—	
* " 29 ...	1759	28 "	1	+	11 "
⊙Nov. 9 ...	1866	30 "	1	+	12 "
" 4 ...	1884	34 "	1	—	
* " 11 ...	1897	36 "	2	+	5 "
* " 16 ...	1907	37 "	1	—	
* " 4 ...	1885	42 "	1	—	
*Oct. 8 ...	1791	46 "	2	+	9 "
*Nov. 3 ...	1881	49 "	1	—	
* " 11 ...	1898	51 "	1	—	
* " 4 ...	1856	53 "	1	—	

Remarks.—In these experiments the intestines only of the infected flies were inoculated. In the experiments marked with an asterisk the gut was first proved, by microscopical examination, to contain swarms of living *Trypanosoma gambiense*, and then immediately injected into a monkey. It will be seen there are 47 experiments in which a total of 296 laboratory-bred flies were inoculated, subcutaneously, into monkeys at various intervals after the flies had fed upon blood infected with *Trypanosoma gambiense*. The injection into a monkey of a single fly, one, two, and four hours after an infected feed, caused Sleeping Sickness. Three flies inoculated two days after their infected feed produced a like result. Between the 2nd and the 24th days after the flies had fed on infected blood, 249 of them were inoculated; the result was negative in every case, although 15 of these flies proved, by microscopical examination, to be swarming with living *Trypanosoma gambiense* at the time of inoculation. In 13 experiments, 14 flies proved, microscopically, to be infected, were inoculated, between the 24th and 53rd days after they had fed upon an infected animal, and in five of these a positive result was obtained.

The following table shows the result of subcutaneous injections of washed salivary glands dissected from laboratory-bred flies whose intestines contained swarms of *Trypanosoma gambiense* :—

Table 22.—*Glossina palpalis* (Salivary Glands) and *Trypanosoma gambiense*.

Date.	Experiment No.	No. of days after infected feed.	No. of flies from which glands injected.	Result.	Incubation period in monkey.
1910.					
Apr. 14 ...	2376	2 days	3	—	
" 15 ...	2376	3 "	1	—	
" 17 ...	2376	6 "	2	—	
" 18 ...	2376	7 "	3	—	
" 22 ...	2394	8 "	2	—	
" 25 ...	2394	11 "	2	—	
Jan. 26 ...	2009	14 "	2	—	
" 31 ...	2114	21 "	1	—	
Feb. 2 ...	2114	23 "	1	—	
" 7 ...	2148	28 "	1	—	
" 9 ...	2148	30 "	1	—	
" 13 ...	2177	36 "	4	+	8 days.

Remarks.—The results of the microscopical examination of the salivary glands show that trypanosomes do not appear in the glands until about the 30th day. Out of 12 experiments 11 were negative, and one was positive. The glands from 19 infected flies were injected, subcutaneously, between the 2nd and 30th days after the flies had fed upon an infected animal; the results were all negative. In the positive experiment the salivary glands from 4 infected flies were injected into a healthy monkey

36 days after the flies had fed on a monkey suffering from Sleeping Sickness, with the result that the former animal contracted the disease.

Conclusions.

1. *Trypanosoma gambiense* may retain its virulence, as ascertained by direct inoculation into susceptible animals, for a period of two days after it has been ingested by *Glossina palpalis*.

2. After the trypanosomes have been within the gut of the fly for two days the power of infecting animals with Sleeping Sickness, when inoculated subcutaneously, is lost for a period of 22 days.

3. *Trypanosoma gambiense* regains the power of infecting by direct inoculation after it has been about 24 days within the intestine of the fly.

4. The number of days, during which the virulence of the trypanosomes contained in the fly is lost, roughly coincides with the time that the infected fly is incapable of transmitting Sleeping Sickness by biting susceptible animals.

5. There is some evidence that the salivary glands of the fly are invaded by virulent forms of the parasite 36 days after the fly has fed upon infected blood.

6.—DURATION OF THE INFECTIVITY OF THE GLOSSINA PALPALIS AFTER THE REMOVAL OF THE LAKE-SHORE POPULATION.

This paper has already been published in the 'Proceedings' of the Royal Society, B, Vol. 82, 1909, p. 56, and reprinted in the Reports of the Sleeping Sickness Commission, No. X., p. 56. The conclusion arrived at was, that the *Glossina palpalis* on the uninhabited shores of Victoria Nyanza can retain their infectivity for a period of at least two years after the native population has been removed.

7.—FURTHER EXPERIMENTS ON THE DURATION OF THE INFECTIVITY OF THE GLOSSINA PALPALIS AFTER THE REMOVAL OF THE LAKE- SHORE AND ISLAND POPULATION.

Experiments have already been published to show that the tsetse flies from the shores of Lake Victoria were capable of infecting animals with Sleeping Sickness up to the end of September, 1909.*

* 'Proceedings' of the Royal Society, B, Vol. 82, 1909, p. 56, and Reports of the Sleeping Sickness Commission, No. X., p. 56.

The following experiments are a continuation of the investigations of the infectivity of *Glossina palpalis* caught on the Lake-shore and on an uninhabited island in the Victoria Nyanza.

(a.)—*To ascertain if Glossina palpalis, caught on the Lake-shore, where there are no Natives, are capable of giving rise to Sleeping Sickness in Healthy Monkeys.*

Experiment 1914.—Monkey.

Date	No. of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1909.			1910.		
Nov. 20	150	—	Feb. 14	150	—
" 22	75	—	" 15	130	—
" 23	50	—	" 16	70	—
" 24	50	—	" 18	50	—
Dec. 2	70	—	" 21	30	—
" 3	60	—	" 22	30	—
" 4	250	—	" 23	40	—
" 6	200	—	" 24	50	—
" 8	25	—	March 1	100	—
" 10	50	—	" 17	45	—
" 13	60	—	" 19	100	—
" 14	35	—	" 29	150	—
" 16	25	—	" 30	100	—
" 27	30	—	April 1	200	—
" 28	30	—	" 4	25	—
" 30	45	—	" 7	100	—
			" 9	100	—
1910.			" 14	140	—
Jan. 6	23	—	" 18	350	—
" 10	50	—	" 19	120	—
" 19	60	—	" 21	90	—
" 20	200	—	" 22	65	—
" 21	65	—	" 25	50	—
" 22	115	—	" 27	50	—
" 25	35	—	" 29	30	—
" 26	50	—	May 7	75	—
Feb. 6	50	—	" 13	Monkey died.	
" 7	50	—			

Remarks.—4,338 flies fed but failed to infect. No trypanosomes ever seen in monkey's blood.

Experiment 1917.—Monkey.

Date.	No. of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1909.			1909.		
Nov. 25	500	—	Dec. 30	35	—
" 30	150	—			
Dec. 2	100	—	1910.		
" 3	100	—	Jan. 6	50	—
" 4	300	—	" 17	80	—
" 6	130	—	" 19	100	—
" 8	35	—	" 20	100	—
" 10	65	—	" 21	100	—
" 13	40	—	" 22	90	—
" 14	55	—	" 25	50	—
" 16	25	—	" 26	50	—
" 18	35	—	" 28	—	+
" 28	40	—			

Remarks.—Result positive. Infection January 21; 2,040 flies fed.

Experiment 1919.—Monkey.

Date.	No. of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1909.			1909.		
Nov. 26	500	—	Dec. 28	30	—
" 27	320		" 30	25	—
Dec. 2	130	—	" 1910.		
" 3	60		Jan. 10	25	—
" 4	200		" 17	30	—
" 6	150	—	" 19	80	
" 8	30		" 22	125	—
" 10	80	—	" 25	30	
" 13	35		" 26	100	
" 14	60		" 28	—	+

Remarks.—Result positive. Infection January 19; 1,755 flies fed.

Experiment 2111.—Monkey.

Date.	No. of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1910.			1910.		
Jan. 28	60	—	Feb. 18	70	
Feb. 6	100		" 21	65	
" 7	100	—	" 22	30	—
" 14	200	—	" 23	50	
" 15	200		" 24	30	—
" 16	100		" 28	150	
" 17	60	—	March 4	—	+

Remarks.—Result positive. Infection February 24; 1,065 flies fed.

Experiment 2112.—Monkey.

Date.	No. of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1910.			1910.		
Jan. 28	180	—	Feb. 16	100	
Feb. 6	100	—	" 17	60	—
" 7	100		" 18	60	
" 14	150	—	" 22	—	+
" 15	150				

Remarks.—Result positive. Infection February 15; 680 flies fed.

Experiment 2215.—Monkey.

Date.	No. of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1910.			1910.		
Feb. 22	30	—	April 1	200	—
" 23	50	—	" 4	100	—
" 24	40	—	" 5	80	—
March 1	100	—	" 7	200	—
" 15	210	—	" 9	100	—
" 18	50	—	" 14	200	—
" 19	200	—	" 15	—	+
" 26	200	—			

Remarks.—Result positive. Infection February 7; 1,460 flies fed.

Experiment 2271.—Monkey.

Date.	No of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1910.			1910.		
March 4	50	—	April 7	200	—
" 15	90	—	" 9	100	—
" 18	50	—	" 14	90	—
" 19	200	—	" 18	250	—
" 23	200	—	" 22	125	—
" 29	150	—	" 25	60	—
" 30	100	—	" 27	90	—
April 1	100	—	" 29	21	—
" 5	90	—	May 6	—	+

Remarks.—Result positive. Infection April 29; 1,966 flies fed.

Experiment 2475.—Monkey.

Date.	No. of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1910.			1910.		
May 14	400	—	June 28	25	—
" 23	200	—	July 15	200	—
" 25	30	—	Aug. 2	260	—
June 2	100	—	" 3	190	—
" 13	250	—	" 4	150	—
" 14	100	—	" 5	190	—
" 15	50	—	Sept. 3	—	Monkey died.

Remarks.—Result negative. 2,145 flies fed but failed to infect.

Experiment 2558.—Monkey.

Date.	No. of flies fed.	Trypanosomes.	Date.	No. of flies fed.	Trypanosomes.
1910.			1910.		
June 14	200	—	July 15	400	—
" 15	70		" 18	67	—
" 28	100	—	" 20	450	
July 1	70	—	" 21	85	—
" 7	190		" 25	300	
" 8	310	—	" 28	100	
" 11	400		" 29	20	—
" 12	150	—	Aug. 1	45	
" 13	300		" 2	—	+
" 14	150				

Remarks.—Result positive. Infection July 25; 3,242 flies fed.

(b.)—*To Ascertain if Glossina palpalis, caught on Damba Island, where there are no Natives, are capable of giving rise to Sleeping Sickness in Healthy Monkeys.*

The population was removed from Damba Island during August and September, 1909. Hippopotami and antelope (*Limnotragus spekei*) were the only large mammals observed on this island. Canoe-men and fly-boys in the service of the Commission visited the island at intervals since November, 1909, for the purpose of obtaining pupæ of *Glossina palpalis*. From the middle of January until the middle of May, 1910, batches of flies were brought from Damba, at intervals of a week or a fortnight, and were fed upon healthy monkeys in the laboratory at Mpumu, with the result shown in the following tables:—

Experiment 2072.—Monkey.

Date.	No. of flies fed.	Trypanosomes.
1910.		
Jan. 14 ...	300	—
Feb. 11 ...	300	—
March 12 ...	150	—
" 21 ...	150	—
" 24 ...	200	—
April 13 ...	110	—
May 6 ...	150	—
" 11 ...	150	—
" 20 ...	150	—
Monkey's blood examined, twice weekly, for one month from May 20.		

Remarks.—Result negative; 1,660 flies fed.

Experiment 2164A.—Monkey.

Date.	No. of flies fed.	Trypanosomes.
1910.		
Feb. 14 ...	200	—
March 12 ...	150	—
„ 21 ...	150	—
„ 24 ...	250	—
April 13 ...	300	—
May 6 ...	300	—
„ 11 ...	300	—
„ 20 ...	170	—
Monkey's blood examined, twice weekly, for one month after May 20.		

Remarks.—Result negative; 1,820 flies fed.

It will be seen that 3,480 flies were fed on two monkeys, with negative results.

During May and June, 1910, the members of the Commission alternately spent a week encamped on Damba, and carried out the feeding experiments on the island. The results are given in the tables below:—

Experiment 2164B.—Monkey.

Date.	No. of flies fed.	Trypanosomes.
1910.		
May 26 ...	120	—
„ 27 ...	120	—
„ 28 ...	60	—
„ 29 ...	150	—
„ 30 ...	100	—
Monkey's blood examined, twice weekly, for one month from May 30.		

Remarks.—Result negative; 550 flies fed.

Experiment 2486.—Monkey.

Date.		No. of flies fed.	Trypanosomes.
1910.			
May 28	...	160	—
" 29	...	230	
" 30	...	200	—
June 10	...	—	+

Remarks.—Result positive. Infection May 30; 590 flies fed.

Experiment 2512.—Monkey.

Date.		No. of flies fed.	Trypanosomes.
1910.			
May 28	...	50	—
" 29	...	200	—
" 30	...	100	—
		Monkey's blood examined, twice weekly, for one month from May 30.	

Remarks.—Result negative; 350 flies fed.

Experiment 2545.—Monkey.

Date.		No. of flies fed.	Trypanosomes.
1910.			
June 6	...	210	—
" 7	...	40	
" 8	...	50	
" 9	...	333	
" 17	...	—	+

Remarks.—Result positive. Infection June 9; 633 flies fed.

Experiment 2547.—Monkey.

Date.		No. of flies fed.	Trypanosomes.
1910.			
June 6	...	70	—
" 7	...	100	—
" 8	...	250	—
" 9	...	333	—
		Monkey's blood examined, twice weekly, for one month from June 9.	

Remarks.—Result negative; 753 flies fed.

A total of 2,876 flies were fed upon five healthy monkeys, *i.e.* an average of 575 flies on each animal. Two of these monkeys became infected with *Trypanosoma gambiense*, thereby proving that the tsetse flies on Damba island were capable of giving Sleeping Sickness to healthy monkeys up to June, 1910.

The following table summarises the results of the experiments recorded in the preceding paper and in the present one:—

Table 23.

Experiment.	Place.	No. of flies fed.	No. of days before infection took place.	Result.	Percentage of infected flies.*
52	... Kibanga ...	91	—	—	—
214	... Uninhabited Lake-shore.	2,500	98	+	0·04
571	... " "	1,002	20	+	0·10
612	... " "	615	12	+	0·16
674	... " "	2,315	29	+	0·04
758	... " "	630	30	+	0·16
976	... " "	440	12	+	0·23
982	... " "	456	16	+	0·22
1117	... " "	525	11	+	0·19
1276	... " "	300	13	+	0·33
1462	... " "	505	10	+	0·19
1465	... " "	295	14	+	0·34
1914	... " "	4,338	—	—	—
1917	... " "	2,040	64	+	0·05
1919	... " "	1,755	63	+	0·06
2111	... " "	1,065	35	+	0·09
2112	... " "	680	25	+	0·15
2215	... " "	1,460	52	+	0·07
2271	... " "	1,966	63	+	0·05
2475	... " "	2,145	—	—	—
2558	... " "	3,242	49	+	0·03
2072	... Damba, an uninhabited island in the Lake.	1,660	—	—	—
2164A	... " "	1,820	—	—	—
2164B	... " "	550	—	—	—
2486	... " "	590	13	+	0·17
2512	... " "	350	—	—	—
2545	... " "	633	11	+	0·16
2547	... " "	753	—	—	—

* This is calculated on the assumption that there is only one infected fly in each batch of flies used in an experiment.

These experiments prove that *Glossina palpalis* on the uninhabited shores of Victoria Nyanza can retain their infectivity for a period of at least three years after the native population has been removed, and that the tsetse flies on the islands may retain their infectivity for at least ten months after removal of the population.

The necessity of continuing these experiments is obvious, since the fertile shores and islands of the Lake will remain uninhabitable so long as these regions are infested with tsetse flies capable of disseminating Sleeping Sickness.

In searching for the reservoir whence the flies acquired the virus, no definite host was discovered on the Lake-shore. Natives, other than our canoe-men and fly-boys, were never observed on the Lake-shores or islands while these experiments were in progress. The canoe-men and fly-boys were kept under careful observation during the whole of the time, their blood constantly examined, and once a month blood from each of them was injected into a healthy monkey, always with a negative result.

It has been proved, however, that antelope are "potential" hosts of the virus of Sleeping Sickness, so that these animals and, possibly, other mammals are probably the source of infection to the fly on the Lake-shore.

8.—MECHANICAL TRANSMISSION OF SLEEPING SICKNESS BY THE TSETSE FLY.*

Up to the beginning of 1909 it was believed that the spread of trypanosome diseases, such as Sleeping Sickness and Nagana, was effected by the mechanical transmission of the parasite by the tsetse fly. The proboscis was supposed to be contaminated by being dipped in the infected blood, and some of the trypanosomes were pictured as remaining for some time within the tube and capable of being injected into a fresh animal at the next feed of the fly. Successful experiments were described which seemed to prove that the tsetse fly was capable of remaining infective for 48 hours, but not longer; and it was thought that any given Sleeping Sickness area would be free from danger a few days after the infected population had been removed from it.

Dr. Kleine, however, at the end of 1908, showed that the tsetse fly remains infective for a much longer period, and that a period of non-infectivity of 20 days or more elapses before this power of passing on the parasite is gained. In other words, that the *Trypanosoma gambiense* undergoes some process of development in the fly before it is able to infect a fresh animal. It was evident, then, that the mechanical theory had to be modified.

It was now held that, in addition to the mechanical method, a mode involving a developmental phase within the fly must be reckoned with. But it was still considered likely that the

* Reprinted with additions from the 'Proceedings' of the Royal Society, B, Vol. 82, 1910.

mechanical was by far the more common mode of infection, and that for every case due to a developmentally-infected fly, a hundred would be due to recent contamination. At the same time, it was quite evident, from an examination of the old feeding experiments after 8, 12, 24, and 48 hours, that the successful results recorded might have to be credited to late-infectivity rather than to mechanical transmission. The experiments lasted so long that there was enough time for the unsuspected late development of the parasite in the fly to have caused the infection.

The following experiments were therefore made to put this matter to the proof. The flies, after having had their infective feed, were, as a rule, not allowed to feed on a healthy animal for more than 12 days, in this way preventing the chance of the infection being transmitted by flies in which the development of *Trypanosoma gambiense* had taken place.

(a.)—*Mechanical Transmission of Trypanosoma gambiense.*
Interrupted Feeding.

In interrupted feeding, the cage containing the flies is first placed on the infected animal for some little time, then suddenly transferred to the healthy animal, and so backwards and forwards for 10 to 15 minutes. This is meant to imitate the conditions in Nature, when sick and healthy natives are sitting together on the Lake-shore, and the tsetse flies are continually flitting from one to the other.

Experiment 1550. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*.
From infected to healthy monkey.

Date.	Day.	Procedure.	Remarks.
1909.			
Aug. 30—31	...	} Cage changed from infected to non-infected monkey every 15 seconds. This continued for 10 minutes each day.	65 flies used.
Sept. 1	...		
" 2	...		Sept. 6, healthy monkey showed <i>Trypanosoma gambiense</i> .
" 3	...		
" 4	...		
" 5	...		
" 6	...		

Result.—Positive. The infection probably took place on the first day (August 30), as it usually takes seven days before the trypanosomes appear in the blood.

Experiment 1316. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*.
Infected ox to healthy monkey.

The two oxen used for this experiment were known to be infective, as their blood, when injected into a clean monkey, gave rise to a *gambiense* infection.

Date.	Day.	Flies fed on infected oxen and healthy monkey.	Alternate feeds.	Remarks.
1909.				
July 15—16 ...	1	Flies fed daily...	20	3 flies used.
" 17—31 ...	2—16	" " ...	20	July 16, 4 flies added.
Aug. 1 ...	17	" " ...	20	Aug. 1, 20 flies added.
" 2—Sept. 7	18—54	" " ...	20	Sept. 7, 10 flies left ; dissected ; all negative. Monkey remained healthy.

Result.—Negative. The trypanosomes are usually very few and far between in the blood of cattle, which probably accounts for the negative result.

Experiment 1543. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*.
Infected monkey to healthy goat.

Date.	Day.	Feedings from infected monkey to healthy goat.	Remarks.
1909.			
Aug. 28 ...	—	20 alternate feeds.	50 flies used.
" 29—30 ...	1	25	
Sept. 9 ...	2—12	Continued daily for 12 days.	

Result.—Positive. The goat, Experiment 1495, was examined daily for trypanosomes until October 16, but none was seen. On October 1, 21 days after the last feed of infected *Glossina palpalis*, 4 c.c. of the blood of this goat were injected into monkey, Experiment 1777. This monkey showed trypanosomes on October 16, proving that the goat, Experiment 1495, had become infected from the monkey by interrupted feeding, though trypanosomes failed to appear in its blood.

Experiment 1565. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*.
Infected ox to monkey.

Date.	Day.	Interrupted feeding on infected ox and healthy monkey.	Remarks.
1909. Aug. 29—Sept. 9...	1—11	25 passages to each animal, the whole lasting about half an hour. This continued daily for 11 days.	30 flies used. The monkey, examined for 30 days, failed to show trypanosomes.

Result.—Negative. Blood of ox injected into monkey on August 28. This monkey became infected with *Trypanosoma gambiense* six days later. The negative result may be due to the small numbers of trypanosomes found at any time in oxen.

Experiment 1705. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*.
Infected monkey to fowl.

Date.	Day.	Interrupted feeding on infected monkey and healthy fowl.	Remarks.
1909. Sept. 28—Oct. 4 ...	1—6	30 passages from monkey to fowl, and continued daily for 6 days.	60 flies used.

Result.—Negative. October 7, 1 c.c. of blood of fowl injected into healthy monkey. November 2, 2 c.c. of blood of fowl injected into healthy monkey. Both monkeys remained healthy.

Table 24.—Summary of Results of Interrupted Feeding Experiments.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*.

Experiment.	Infected Animal.	Healthy Animal.	No. of days flies fed.	Result.		Remarks.
				Positive.	Negative.	
1550	Monkey	Monkey	7	+	—	65 flies used.
1316	Ox ...	Goat ...	54	—	—	5 to 29 flies used.
1543	Monkey	Goat ...	12	+	—	50 flies used.
1565	Ox ...	Monkey	11	—	—	30 flies used.
1705	Monkey	Fowl ...	6	—	—	60 flies used.

(b.)—*Mechanical Transmission of Trypanosoma gambiense by Laboratory-bred Glossina palpalis, with an Interval between the Feeding of the Flies on the Infected and Healthy Animals.*

The following table gives a summary of the results:—

Table 25.

Experiment.	No. of days flies fed.	No of flies fed.	Infected animal.	Healthy animal.	Interval between feedings.	Result.	
						Positive.	Negative.
2039	6	170	Monkey	Monkey	10 mins.	+	
2311	1	80	"	"	10 "	+	
2312	2	80	"	"	10 "	+	
2123	6	10	"	"	10 "		—
2318	2	10	"	"	10 "		—
2042	6	100	"	"	20 "	+	
2124	6	10	"	"	20 "		—
1712	12	50	"	"	$\frac{1}{2}$ hour		—
2043	6	100	"	"	$\frac{1}{2}$ "		—
2315	1	10	"	"	$\frac{1}{2}$ "		—
2122	6	120	"	"	40 mins.	+	
2181	6	60	"	"	1 hour	+	
2309	1	60	"	"	1 "	+	
2323	4	100	"	"	1 "	+	
1233	12	14	"	"	1 "		—
1664	12	50	"	"	1 "		—
2057	6	100	"	"	1 "		—
2174	6	200	"	"	1 "		—
2314	2	100	"	"	1 "		—
2321	3	100	"	"	1 "		—
2230	8	200	"	"	$1\frac{1}{2}$ hours		—
2354	6	200	"	"	$1\frac{1}{2}$ "		—
2355	4	150	"	"	$1\frac{1}{2}$ "		—
2361	4	200	"	"	2 hours	+	
1665	12	40	"	"	2 "		—
1524	11	35	"	"	6 "		—
1080	12	11	"	"	8 "		—
2525	5	200	"	"	18 "		—
1801	13	70	"	"	24 "		—
1802	13	75	"	"	24 "		—
1803	13	120	"	"	24 "		—
2524	7	150	"	"	24 "		—
1274	15	7	"	"	48 "		—

Conclusions.

1. The mechanical transmission of Sleeping Sickness by means of *Glossina palpalis* can take place if the transference of the flies from the infected to the healthy animal is instantaneous—that is, by interrupted feeding.

2. This mechanical transmission can also take place if the interval of time of the transference of the flies from the infected to the healthy animal is not longer than two hours.

3. Mechanical transmission plays a much smaller part, if any, in the spread of Sleeping Sickness than has been supposed.

B. EXPERIMENTS DESIGNED TO DISCOVER THE SOURCE OF THE INFECTION OF SLEEPING SICKNESS.

9.—EXPERIMENTS TO ASCERTAIN IF CATTLE MAY ACT AS A RESERVOIR OF THE VIRUS OF SLEEPING SICKNESS (*Trypanosoma gambiense*).*

The question as to whether cattle can act as a reservoir of the virus of Sleeping Sickness is an important one. It was usually believed until lately that man was the main reservoir, and that the other animals might be ignored. But in view of the fact that the flies on the Lake-shore have remained infective for some three years after the native population have been removed, it is necessary to inquire if it is not possible that other animals may act as well.

In this regard cattle have been, perhaps, the most important, as on the once thickly populated Lake-shore and islands they were numerous, and in many cases grazed and watered in the fly-area. Another reason of their importance is, that if they can act as a reservoir, then the same will probably be true of the different species of antelope which inhabit the Lake-shore. It may be presumed that these will greatly increase now that the natives and domestic animals have been removed, and that they will take the place of the cattle in keeping up the infectivity of the *Glossina palpalis*.

The Commission, therefore, thought it would be well to inquire into the question, and the result is given in this paper.

Answers to the following questions were sought: Are cattle capable of being infected with Sleeping Sickness by the subcutaneous injection of blood containing *Trypanosoma gambiense*? Can cattle be infected with Sleeping Sickness by the bites of artificially-infected tsetse-flies? Can cattle be infected with Sleeping Sickness by the bites of the naturally-infected flies caught on the Lake-shore? Is it possible to infect tsetse-flies by feeding them on cattle infected with Sleeping Sickness, and afterwards to transmit the disease by means of these flies to healthy animals? Finally, if these questions are answered in the affirmative, will it be possible to find that cattle which have lived in the fly-area are naturally infected with Sleeping Sickness?

(a.)—*Are Cattle capable of being Infected with Sleeping Sickness by the Subcutaneous Injection of Blood containing Trypanosoma gambiense?*

Experiment 869. Bull.

September 10, 1909.—A bull was inoculated with 5 cc. of blood containing large numbers of *Trypanosoma gambiense* from an infected monkey.

Its blood was examined daily, and 18 days after injection the bull was found to be infected with *Trypanosoma gambiense*. The identity of the trypanosome was established by injecting a monkey with some blood from the ox. This monkey showed *Trypanosoma gambiense* on the sixth day.

* Reprinted from the 'Proceedings' of the Royal Society, B, Vol. 82, 1910.

Conclusion.—From this experiment it is seen that oxen are capable of being infected with Sleeping Sickness by the injection of blood containing *Trypanosoma gambiense*. The trypanosome appears in small numbers in the blood, and the blood, when injected into susceptible animals such as monkeys, gives rise to a fatal form of the disease.

(b.)—*Can Cattle be Infected with Sleeping Sickness by the Bites of Artificially-Infected Glossina palpalis?*

The two following experiments were carried out by feeding *Glossina palpalis* first on an infected monkey, and immediately afterwards on a healthy ox. Wild flies from the Lake-shore were used.

Experiment 890. Ox.

May 20, 1909.—The ox was thrown and a monkey heavily infected with Sleeping Sickness was laid across its flank. Two cages of *Glossina palpalis*, containing 100 and 150 flies respectively, were allowed to feed for a few seconds on the monkey and then on the ox. The flies were allowed from 30 to 35 interrupted feeds on each animal every day. This was continued for 38 days, during which time 561 flies were estimated to have fed on one or other animal.

July 17.—Fifty-eight days after the first infected feed *Trypanosoma gambiense* appeared in the blood of the ox.

The identity of the trypanosome was established by injection of the ox's blood into two monkeys. The first monkey was injected with blood from the ox 76 days, and the second monkey 181 days after the flies had first fed on the ox. Both monkeys developed Sleeping Sickness, the first 7 days and the second 11 days after injection of the blood.

Experiment 891. Calf.

The details of this experiment were similar to those of the last. *Trypanosoma gambiense* appeared in the blood of the calf 57 days after the flies had been first fed upon it.

Three cubic centimetres of the blood of the calf were injected into a monkey, and the monkey developed Sleeping Sickness after an incubation period of 8 days.

Conclusion.—These two experiments show that when artificially-infected *Glossina palpalis* are allowed to feed on healthy cattle, these animals develop Sleeping Sickness, and that the blood of the cattle is capable of giving rise to infection of *Trypanosoma gambiense* in monkeys when injected into them.

(c.)—*Can Cattle be Infected with Sleeping Sickness by the Bites of the Naturally-Infected Flies caught on the Lake-shore?*

In the next three experiments freshly caught *Glossina palpalis* brought up to the laboratory from the Lake-shore were allowed to feed straightway on healthy cattle. By this means it will be shown whether *Glossina palpalis* in their wild state are capable of giving Sleeping Sickness to healthy cattle.

Experiment 982. Bull.

2,195 freshly captured *Glossina palpalis* were applied to a bull, and of these 1,536 were estimated to have fed. This feeding of the flies extended over a period of 16 days, at the end of which time *Trypanosoma gambiense* appeared in the blood of the bull.

To help in the identification of this trypanosome, 3 cc. of the blood of the bull were injected into a monkey. The monkey developed Sleeping Sickness 18 days later. 5 cc. of the blood of the bull were also injected into a goat. *Trypanosoma gambiense* appeared in the blood of the goat after an incubation period of 38 days.

Experiment 1,462. Bull.

The details of this experiment were similar to those of the last one. Over a period of 8 days 1,370 wild flies from the Lake-shore were applied to the bull, of which 705 fed. Ten days from the first application of flies *Trypanosoma gambiense* appeared in the blood of the bull.

Two animals, a monkey and a goat, each received 1 cc. of the blood of the bull by injection under their skin. The monkey developed Sleeping Sickness seven days later, but the goat died in 16 days without showing any infection.

Experiment 1,465. Bull.

During a period of 13 days, 459 freshly caught Lake-shore *Glossina palpalis* were applied to a bull, and of these 314 fed. On the 14th day after the flies were first fed the bull developed infection of *Trypanosoma gambiense*.

Some blood from this bull was injected into a monkey and into a goat. Neither animal became infected.

Conclusion.—These experiments prove that *Glossina palpalis*, when captured in their natural state on the Lake-shore, are capable of transmitting the virus of Sleeping Sickness to cattle, and that the blood of these cattle gives rise to a fatal form of the disease in monkeys and in goats when it is injected into them.

(d.)—*Is it possible to Infect Tsetse-flies by Feeding them on Cattle Infected with Sleeping Sickness, and afterwards to Transmit the Disease by means of these Flies to Healthy Animals?*

Five experiments under this heading were carried out. Laboratory-bred flies were used in all of them. Three were negative and two positive. The three negative experiments will be shortly summarised first.

Experiment 1,451.

Ninety laboratory-bred *Glossina palpalis* were fed for 10 successive days on a calf whose blood contained *Trypanosoma gambiense*. The flies were starved for 72 hours. They were then fed on a clean monkey daily for 45 successive days. The monkey failed to develop Sleeping Sickness.

When the remainder of the flies were dissected, one contained flagellates, but when the contents of this fly were injected into

a goat the animal failed to show any infection of *Trypanosoma gambiense*.

Result.—Negative.

Experiment 1,269.

The details of this experiment were similar to those of the last. After the *Glossina palpalis* had been fed on two oxen whose blood contained *Trypanosoma gambiense*, they were applied daily to a monkey. They were fed on this monkey for 35 consecutive days and were then transferred to a second monkey. Both the monkeys remained healthy.

Two of the flies were found on dissection to contain flagellates, but when these were injected into a monkey and a goat no development of Sleeping Sickness took place in these animals.

Result.—Negative.

Experiment 1,672.

Here again the technique was similar to the last. The *Glossina palpalis* were fed on alternate days for a lengthened period, on a clean monkey and a clean goat. Both animals remained healthy.

Some infected flies were found on dissection, but when introduced under the skin of a goat and of a monkey did not give rise to Sleeping Sickness.

Result.—Negative.

The next two experiments, which were carried out in the same way as the two preceding ones, were positive.

Experiment 1,566.

The *Glossina palpalis* were fed on an infected ox, and after a starve of 72 hours were fed on a clean monkey for 45 successive days. Sixty-eight days after the flies had taken their first infected feed this monkey developed Sleeping Sickness.

When the flies came to be dissected nine of them showed flagellates either in the proboscis or in the alimentary tract. Some of these were injected into goats and into a monkey, but with negative results.

Result.—Positive.

Experiment 1,602.

Fifty laboratory-bred flies were fed for four successive days on an ox whose blood contained *Trypanosoma gambiense*. After a period of starvation they were applied to a monkey and to a goat on alternate days. The monkey died before it could have become infected, but the goat developed Sleeping Sickness 20 days after the flies had their first infected feed.

The remainder of the flies, 32 in all, were dissected, and five were found to contain flagellates. The alimentary contents of one of these flies were injected into a monkey, and after an incubation period of 13 days *Trypanosoma gambiense* appeared in its blood.

Result.—Positive.

Conclusion.—Laboratory-bred tsetse-flies can be infected by feeding them on cattle infected with Sleeping Sickness, and afterwards the disease can be transmitted to healthy animals by means of these flies.

(e.)—Do Cattle, when Living in the Fly-area, actually carry the Virus of Sleeping Sickness?

About seventeen cattle from various sources were examined with this point in view. Not all these cattle could be proved to have been exposed to the bites of *Glossina palpalis*, but most of them came from places where these flies are plentiful. One was positive.

Experiment 1,633.

This cow came from the island of Kome, in Lake Victoria, where human Sleeping Sickness is prevalent and where *Glossina palpalis* abound.

Trypanosoma gambiense was found in its blood by microscopical examination, and when 3 cc. of the blood were injected under the skin of a monkey the animal developed Sleeping Sickness after an incubation period of seven days.

Conclusion.—This experiment proves that cattle in their natural state, and apparently in good health, may harbour the virus of Sleeping Sickness.

Conclusions.

It has been proved by experiment that cattle may act as a reservoir of the virus of Sleeping Sickness, and that healthy animals may be infected from them by means of *Glossina palpalis*.

It has also been proved that cattle in the fly-area do naturally harbour *Trypanosoma gambiense*.

It is, therefore, possible that the cattle and antelope living in the fly-area may act as a reservoir, and so keep up the infectivity of the *Glossina palpalis* for an indefinite period, but there is no proof up to the present that this actually takes place in Nature.

10.—EXPERIMENTS TO ASCERTAIN IF ANTELOPE MAY ACT AS A RESERVOIR OF THE VIRUS OF SLEEPING SICKNESS (*Trypanosoma gambiense*).*

The question of a reservoir of the virus of Sleeping Sickness, other than man and his domestic animals, is of the utmost importance.

Now man and the domestic animals have been removed from the Lake-shore of the mainland for some two and a-half years, and from the islands since September, 1909. The effect of this depopulation has been to make a two-mile area along the northern shores of the Lake virtually a game reserve, in which water-buck, bush-buck, reed-buck, Speke's *Tragelaphus*, hippopotami, wild pig, and other large game abound. The game drink freely at the Lake-shore, and small herds of antelope may frequently be seen grazing on the grassy hillsides overlooking the Lake.

Notwithstanding the removal of man and his domestic animals, the Lake-shore *Glossina palpalis* continued to infect susceptible animals with Sleeping Sickness up to the end of July, 1910. The Commission, therefore, endeavoured to find answers to the following questions:—

1. Can antelope be infected with Sleeping Sickness by the bites of laboratory-bred and laboratory-infected *Glossina palpalis*?

* Reprinted from the 'Proceedings' of the Royal Society, B, Vol. 83, 1911.

2. If antelope can be infected with the virus of Sleeping Sickness, can they transmit the infection to laboratory-bred *Glossina palpalis* when these flies are allowed to feed upon them? Further, if these *Glossina palpalis* become infected, can they transmit the virus to susceptible animals?

3. If these *Glossina palpalis* become infected with the virus of Sleeping Sickness, what percentage are so infected?

4. How does Sleeping Sickness affect the health of the antelope?

5. Lastly, are antelope living in the fly-area naturally infected with Sleeping Sickness?

Should all these questions be answered in the affirmative, the importance of the observation is patent. The continued infectivity of the flies on the Lake-shore would be explained. Whilst the movements of man and the domestic animals can, to some extent, be controlled by administrative measures, the movements of antelope in search of new grazing grounds would be almost impossible to check, owing to the difficult nature of the forested and elephant-grass country which in so many parts borders the Uganda shores of the Lake. Game laws would require modification, and the destruction of the game in the neighbourhood of the lakes and *palpalis*-frequented rivers would become a factor in the control of Sleeping Sickness.

To answer the above queries the following experiments were devised and carried out:—

1. *Can Antelope be Infected with Sleeping Sickness by the Bites of Laboratory-bred and Laboratory-infected Glossina palpalis?*

Eleven buck in all were employed in this experiment. Four bush-buck (*Tragelaphus sylvatica?*), six reed-buck (*Cervicapra arundinacea*), and one water-buck (*Cobus defassa*). Other buck, such as “oribi” (*Cephalophus grimmii*) and “entalaganya” (*Cephalophus equatorialis*), were obtained by the Commission, but did not survive long in captivity.

As each buck arrived its blood was usually injected, subcutaneously, into monkeys or rats, to ascertain if the blood was naturally infected with trypanosomes. The method then adopted in each case to infect the antelope with Sleeping Sickness was as follows: A cage of clean* laboratory-bred *Glossina palpalis* was fed on a monkey known to be infected with a human strain of *Trypanosoma gambiense*. This cage of artificially-infected flies was then fed on healthy animals until one of the animals (monkeys) became infected with Sleeping Sickness. Having proved that the flies were infective, the cage of flies was then fed, about five minutes daily, for several days, on one or other of the buck. The buck's blood was then examined daily for trypanosomes, and was further tested for Sleeping Sickness by its inoculation into monkeys or rats.

The experiments follow in full detail.

* The word “clean,” applied here and throughout this Report to laboratory-bred *Glossina palpalis*, means flies which have newly hatched out from pupæ in the laboratory and have never fed.

Experiment 2328. Bush-buck.

This bush-buck was fed on for five days (March 24, 25, 26, 28, and 29, 1910) by laboratory-bred *Glossina palpalis* which were known to be infected with a human strain of *Trypanosoma gambiense*.

On April 8, 15 days after the first feed of the infected flies on this buck, 5 c.c. of the buck's blood were injected, subcutaneously, into a healthy monkey.

April 19, monkey showed *Trypanosoma gambiense* in its blood. The trypanosomes were verified by examination in fresh and stained preparations of the blood.

Result.—Positive.

Remarks.—The preliminary inoculation of the blood into susceptible animals, to ascertain if the buck's blood naturally harboured trypanosomes, was not made in this case. Though examined almost daily from March 29 to August 5, 1910, *Trypanosoma gambiense* was never seen in the blood of this antelope. Fresh and stained blood films were made and examined. On one occasion only—April 20—*Trypanosoma ingens* was seen in a fresh preparation. Monkeys are not inoculable with this parasite.

Experiment 2357. Reed-buck.

April 8, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy, no trypanosomes appearing in its blood.

Buck was fed on for 12 days, between April 7 and 20, 1910, by laboratory-bred *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*. On the 13th day after the first feed of these flies, 3 c.c. of the buck's blood were inoculated into a normal monkey. Examined bi-weekly for a month this monkey remained healthy.

Trypanosoma gambiense having appeared in the buck's blood on May 2, a further inoculation of 5 c.c. of its blood was made into a normal monkey on this date. This monkey showed *Trypanosoma gambiense* in its blood on May 10.

Result.—Positive.

Remarks.—This reed-buck was evidently free from any trypanosome infection inoculable into monkeys on its arrival at the laboratory. *Trypanosoma gambiense* appeared for the first time in the buck's blood in scanty numbers on May 2, 1910, 25 days after the first feed of the supposed infective *Glossina palpalis*. On May 3 and 4, the trypanosomes were fairly numerous in the blood, and on the 5th and 6th, scanty numbers only were seen. After May 6, *Trypanosoma gambiense* was never again seen in the blood, though examined for almost daily up to August 5, 1910. The long period of 25 days which elapsed between the first feed of the supposed infected *Glossina* and the appearance of the trypanosomes in the buck's blood may be accounted for by the supposition that the infected fly or flies in the cage which was fed on the buck from April 7 to April 19 had died before

they had fed on the buck. On April 20, a fresh cage of *Glossina palpalis*, known to be infected with a human strain of *Trypanosoma gambiense* was fed, once only, on this buck, and on the 12th day after this feed, on May 2, trypanosomes appeared in the buck's blood. This supposition is probably correct, and further accounts for the failure of the inoculation on April 20.

Experiment 2359. Reed-buck.

On April 8, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy.

Buck was fed on for six days (April 25-30, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*.

On May 6, the 11th day after flies' first feed, *Trypanosoma gambiense* appeared in buck's blood in fair numbers, and 5 minims were injected, subcutaneously, into normal white rat. On May 11 this rat showed *Trypanosoma gambiense* in its blood.

Result.—Positive.

Remarks.—This reed-buck was evidently free from any trypanosome infection inoculable into monkeys on its arrival at the laboratory. *Trypanosoma gambiense* appeared for the first time in its blood in fair numbers on May 6, 1910, again on 7th, not examined for on 8th, scanty on 9th, absent on 10th, scanty on 11th, very numerous on 12th, and never seen again, though examined for almost daily, till August 5.

Experiment 2371. Bush-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy.

Buck was fed on for eight days (April 22 and 23, and from April 25 to 30, 1910, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*.

On May 4, the 12th day after the infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in the buck's blood in fair numbers.

On May 5, trypanosomes were numerous, and 2 c.c. of the buck's blood were injected, subcutaneously, into a normal monkey. On May 13 this monkey showed *Trypanosoma gambiense* in its blood.

Result.—Positive.

Remarks.—Buck was free from trypanosomes inoculable into a monkey on its arrival at the laboratory. *Trypanosoma gambiense* appeared in buck's blood for the first time, in scanty numbers, on May 3, 1910, and again in fair numbers on May 4; thereafter no trypanosomes were seen up to August 5, 1910, though the blood was almost daily examined.

Experiment 2378. Water-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if the buck naturally harboured trypanosomes in its blood. This monkey was examined bi-weekly for one month; monkey remained healthy.

Buck was fed on for eight days (April 22, 23, 25 to 30, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*. *Trypanosoma gambiense* was never seen in this buck's blood, though examined for almost daily from April 22 to August 5, 1910.

On May 5, 5 c.c. of the buck's blood were injected, subcutaneously, into a normal monkey, an interval of 13 days having elapsed since first feed of the infected flies. This monkey showed *Trypanosoma gambiense* in its blood on May 13, 1910.

Result.—Positive.

Remarks.—Buck was free from trypanosomes inoculable into a monkey on its arrival at the laboratory. Though *Trypanosoma gambiense* never appeared in the buck's blood, yet a positive result was obtained on its inoculation into a monkey, and, as will be seen below (Table 27), clean laboratory-fed flies fed on this buck for several days became infected with *Trypanosoma gambiense*.

Experiment 2427. Reed-buck.

On May 4, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. This rat, examined bi-weekly, for one month, remained healthy.

Buck was fed on for six days (May 2 to 7, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*.

On May 9, the seventh day after infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in scanty numbers in its blood for the first time. One cubic centimetre of the blood was then injected, subcutaneously, into a normal white rat. On May 16, *Trypanosoma gambiense* appeared in this rat's blood.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into a rat. A few *Trypanosoma gambiense* appeared for the first time on May 9, 1910, in buck's blood; they were present in fair numbers on the 10th, scanty again on the 11th, and were seen for the last time, in fair numbers, on May 12. Almost daily blood examinations were made, with negative results, up to August 5, 1910.

Experiment 2428. Bush-buck.

On May 4, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. This rat remained healthy, its blood being examined for one month after the injection.

Buck was fed on for 13 days (May 2-16, inclusive, May 9 and 15 being excepted) by laboratory-bred *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*.

Trypanosoma gambiense was never seen in this animal's blood, though examined for almost daily from May 4 to August 5, 1910.

On May 16, 14 days after infected flies' first feed on the buck, a few drops of the buck's blood were injected, subcutaneously, into a normal white rat. This rat showed *Trypanosoma gambiense* in its blood on May 23. No trypanosomes were ever seen in buck's blood.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into a rat. No trypanosomes were ever seen in the buck's blood, nevertheless its blood was infective on inoculation, and, as will be seen later (Table 27), capable of infecting clean laboratory-bred *Glossina palpalis*.

Experiment 2429. Reed-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey. This monkey remained healthy, its blood being examined bi-weekly for a month after the injection. On May 3, 1910, 1 c.c. of the buck's blood was injected, subcutaneously, into a normal rat. This rat remained healthy, its blood also being examined bi-weekly for a month after the injection.

The buck was fed on for eight days (May 2-7, and 9 and 10, inclusive) by *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*.

On May 11, the ninth day after the infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in scanty numbers in its blood for the first time. On May 13, the trypanosomes being numerous in the blood of the buck, a few drops of the blood were injected, subcutaneously, into a normal white rat. This rat showed *Trypanosoma gambiense* in its blood on May 17.

On July 2, 1910, this buck accidentally broke its leg and had to be killed, 4 c.c. of its heart's blood being injected into a normal monkey. This monkey showed *Trypanosoma gambiense* in its blood on July 12.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into monkeys or rats. *Trypanosoma gambiense* appeared in its blood for three days—on May 11 for the first time in scanty numbers, on May 12, many, and on May 13, very many—thereafter no trypanosomes were seen, though almost daily examinations were made up to July 2, 1910. It will be noted that the buck's blood was still infected with *Trypanosoma gambiense* on July 2—that is, 50 days after the last date (May 13) that trypanosomes were seen in it.

Experiment 2431. Reed-buck.

On May 3, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. Rat remained healthy, its blood being examined bi-weekly for a month after the injection.

The buck was fed on for six days (May 2-7, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*.

On May 12, 1910, the tenth day after the infected flies' first feed, *Trypanosoma gambiense*, in scanty numbers, appeared for the first time in the blood of the buck. On May 13, the trypanosomes being numerous in the blood of the buck, a few drops of its blood were injected, subcutaneously, into a normal white rat. This rat showed numerous *Trypanosoma gambiense* in its blood on May 20.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into rats. *Trypanosoma gambiense* appeared in the buck's blood, in scanty numbers, for the first time on May 12, and were present in large numbers on May 13 and 14.

We have now to record one of the most important and suggestive observations in this series of experiments. *From May 14 to June 27 the blood was examined almost daily, and on the latter date—June 27—44 days after trypanosomes were last seen, Trypanosoma gambiense reappeared for one day in this buck's blood in fair numbers.* Their identity was established by careful examination in wet and stained preparations of the blood.

(N.B.—Two mechanical transmission experiments were carried out with this buck. In the first, 50 flies were used and were fed on the buck for the three days the trypanosomes were seen in its blood. *Result.*—Negative. In the second, 100 flies were used, and were fed for four days on the buck. No trypanosomes were seen in the buck's blood during these days. *Result.*—Negative. In the first experiment four hours, and in the second one hour, elapsed between the feed on the buck and the feed on the healthy monkey.)

Experiment 2445. Reed-buck.

No preliminary inoculation of this buck's blood was made on its arrival at the laboratory.

The buck was fed on for seven days (May 6 and 7, and 9-13, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a human strain of *Trypanosoma gambiense*.

On May 14, 1910, the eighth day after the infected flies' first feed, *Trypanosoma gambiense* appeared in the blood of the buck for the first time. On May 20 the buck died, and a small quantity of its heart's blood was injected, subcutaneously, into a normal white rat. On June 2 rat showed *Trypanosoma gambiense* in its blood. (This rat was overlooked from May 24 to June 2, and its blood was not examined between these dates.)

Result.—Positive.

Remarks.—The blood of this buck was (with the exception of May 8) examined daily from May 6 to 13, with negative results, for trypanosomes. On May 14, *Trypanosoma gambiense* appeared for the first time in its blood in scanty numbers; on the 16th, 17th, and 18th they were very numerous; on the 19th

they were again scanty, and on the day of death, May 20, 1910, no trypanosomes could be found, the peripheral and heart's blood being examined.

Table 26 gives results of feeding infected *Glossina palpalis* on healthy antelope:—

Table 26.

No. of experiment.	Species of antelope.	No. of days infected flies fed.	No. of days before trypanosomes appeared.	Result.		Remarks.
				Posi- tive.	Nega- tive.	
2328	Bush-buck	5	—	+		Trypanosomes never seen.
2357	Reed-buck	12	25	+		
2359	"	6	11	+		
2371	Bush-buck	8	12	+		Trypanosomes never seen.
2372	"	6	8	+		
2378	Water-buck	8	—	+		
2427	Reed-buck	6	7	+		Trypanosomes never seen.
2428	Bush-buck	13	—	+		
2429	Reed-buck	8	9	+		
2431	"	6	10	+		
2445	"	7	8	+		

From these experiments it is shown that antelope may be readily infected with Sleeping Sickness by the bites of artificially-infected tsetse flies. Eleven antelope were used, and in every case a positive result was obtained. It will be remembered that in similar experiments made with cattle the same result was obtained.

2. *If Antelope can be Infected with the Virus of Sleeping Sickness, can they Transmit the Infection to Clean Laboratory-bred Glossina palpalis when these Tsetse Flies are allowed to Feed upon Them? Further, if these Glossina palpalis become infected, can they transmit the Virus to Susceptible Animals?*

It has now been proved that water-buck, reed-buck, and bush-buck can be infected with the virus of Sleeping Sickness, with what would seem to be unfailing regularity. Should, however, these antelope be incapable of infecting the *Glossina palpalis* with *Trypanosoma gambiense*, the fact is of academic importance only. On the other hand, should the results of feeding clean laboratory-bred *Glossina palpalis* on these infected buck give positive results when these flies are subsequently fed on normal susceptible animals, a further step has been made in the search for a reservoir of the virus of Sleeping Sickness, other than man and his domestic animals.

The method adopted to test this second query was carried out as follows:—Clean, laboratory-bred *Glossina palpalis* were fed for

several days on an infected buck. After an interval of starvation of 24 hours or more the flies were transferred to healthy animals and fed daily. When the healthy animal showed *Trypanosoma gambiense* in its blood the experiment was stopped, and the surviving flies were dissected as soon as possible.

The result of 24 experiments carried out on these lines is given in the following table:—

Table 27.—Giving the Result of Feeding Laboratory-bred Flies on Antelope Infected with Sleeping Sickness.

No. of experiment.	No. of clean flies used.	Species of antelope flies fed on.	No. of days flies fed on antelope.	No. of days before flies became infective.	Result.		Remarks.
					Positive.	Negative.	
2346	160	Bush-buck 2328.	12	29	+		Buck 2328 never showed <i>T. gambiense</i> in blood. In spite of this, flies fed on it became infected 55 days after the buck's infection.
2384	100	"	8	28	+		
2414	70	"	6	29	+		
2501	100	"	8	39	+		
2351	100	Reed-buck 2357.	7	41	+		Buck 2357 showed <i>T. gambiense</i> in its blood for 5 days only.
2500	100	"	8	—		—	
2510	100	"	5	—		—	
2507	200	Reed-buck 2359.	6	44	+		
2421	50	Bush-buck 2371.	6	—		—	Buck 2371 showed <i>T. gambiense</i> in its blood for 3 days only.
2477	60	"	6	29	+		
2499	100	Bush-buck 2372.	8	—		—	
							Buck 2372 showed <i>T. gambiense</i> in its blood for 2 days only.
2451	95	Water-buck 2378.	6	30	+		Buck 2378 never showed <i>T. gambiense</i> in its blood.
2478	60	"	6	—		—	
2559	50	"	4	—		—	
2454	110	Reed-buck 2427.	6	24	+		
2456	60	"	4	33	+		Buck 2427 showed <i>T. gambiense</i> in its blood for 4 days only.
2508	100	"	6	30	+		
2485	50	Bush-buck 2428.	7	28	+		
							Buck 2428 never showed <i>T. gambiense</i> in its blood.
2460	50	Reed-buck 2429.	4	27	+		Buck 2429 showed <i>T. gambiense</i> in its blood for 3 days only.
2543	100	"	6	49	+		
2464	55	Reed-buck 2431.	3	28	+		Buck 2431 showed <i>T. gambiense</i> in its blood for 4 days only. In spite of this, flies fed on it became infected 81 days after its infection.
2544	90	"	6	36	+		
2592	100	"	5	43	+		
2476	50	Reed-buck 2445.	4	—		—	Buck 2445 showed <i>T. gambiense</i> in its blood for 6 days only.

On glancing at Table 27 it will be seen that 24 experiments in all were carried out. Of these, 17 were positive (70·84 per cent.), and seven negative (29·16 per cent.). The shortest time which elapsed before the flies became infective was 24 days, the longest 49 days, and the average 33·35 days. Compare these results with those detailed in the 'Proceedings' of the Royal Society, B, vol. 82, 1910, p. 374, Table III.* Of the 42 experiments there described, only 8 (19 per cent.) were positive. The clean laboratory-bred flies were fed on *Trypanosoma gambiense*-infected monkeys in 36 of those experiments, in one case on a Sleeping Sickness patient, and in five cases on oxen infected with the virus of Sleeping Sickness.

Positive results were obtained from all the buck on at least one occasion, with the exception of bush-buck, Experiment 2372, and reed-buck, Experiment 2445. Only two experiments were carried out from these buck, one from each, viz., Experiments 2499 and 2476. In Experiment 2499 the flies were fed on the antelope 19 days after the trypanosomes had disappeared from its blood, as far as microscopical examination went. In Experiment 2476 the flies were non-infective to monkeys up to the 45th day after their first feed on the infected buck. This latter experiment was proceeding when the Commission left Uganda, and a positive result may yet have to be recorded.

The most significant of the above observations is the one in which it is shown that 55 days after the last feed of infected *Glossina palpalis* on bush-buck, Experiment 2328, the blood of this buck was capable of infecting clean laboratory-bred flies, though *Trypanosoma gambiense* had never been seen in its blood.

To illustrate how these experiments were carried out, full details of two are given. They are typical of the methods adopted. One positive and one negative experiment have been chosen. (See next page.)

These experiments show that antelope of the water-buck, reed-buck, and bush-buck species, when infected with the virus of Sleeping Sickness, can transmit the infection to clean laboratory-bred *Glossina palpalis*. The infected antelope's blood was, in one case, infective to *Glossina palpalis* for at least 81 days, and in another for at least 55 days. These experiments further show that the flies, when infected by the virus of Sleeping Sickness obtained from the blood of infected antelope, are capable of transmitting the virus to susceptible animals.

* Reprinted at p. 18 of this Report, Table 3.

Experiment 2501.—To ascertain if Laboratory-bred *Glossina palpalis* become Infective when Fed on Antelope whose Blood contains *Trypanosoma gambiense*.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. May 23—28	1—5	Flies fed 5 minutes daily on Bush-buck 2328.		100 flies used.
„ 29 ...	6	Flies starved.		
„ 30—31	7—8	Flies again fed 5 minutes daily on Buck 2328.		
June 1 ...	9	Flies starved.		
„ 2— July 7	10—45	Flies fed 5 minutes daily on normal monkey 2517.		July 8, Monkey 2517 shows <i>T. gambiense</i> in its blood to-day.
July 8 ...	46	Flies starved, as Monkey 2517 shows <i>T. gambiense</i> in its blood.	+	Allowing 7 days for incubation of <i>T. gambiense</i> in monkey's blood, then the <i>G. palpalis</i> became infective on the 39th day after their first infected feed on Buck 2328.
„ 9 ...	47	The 57 surviving <i>G. palpalis</i> dissected; 20·3 per cent. of these flies showed heavy intestinal infection with flagellates (<i>T. gambiense</i>)		

Remarks.—This is the experiment referred to above, where the blood of a buck was capable of infecting clean laboratory-bred flies 55 days after last feed of infected flies on the buck.

Experiment 2499.—To ascertain if Laboratory-bred *Glossina palpalis* become Infective when Fed on Antelope whose Blood contains *Trypanosoma gambiense*.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. May 23—28	1—5	Flies fed 5 minutes daily on Bush-buck 2372.		100 flies used.
„ 29 ...	6	Flies starved.		
„ 30—31	7—8	Flies again fed 5 minutes daily on Buck 2372.		
June 1 ...	9	Flies starved.		
„ 2—11	10—19	Flies fed on Cock 2518.		
„ 12 ...	20	Flies starved.		
„ 13— July 12	21—50	Flies fed 5 minutes daily on clean Monkey 2552.	—	Monkey 2552 was examined bi-weekly from June 13 to August 12. It remained healthy. The surviving <i>G. palpalis</i> were not dissected as experiment was negative.
July 13 and following days.	51	Flies allowed to die. Not dissected as experiment negative.		

3. *If Glossina palpalis can be infected with the Virus of Sleeping Sickness by Feeding on the Blood of Trypanosoma gambiense-infected Antelope, what Percentage are found to be so Infected?*

It has been shown now that antelope can be infected with the virus of Sleeping Sickness, that when so infected they can infect the fly, and the fly in its turn can convey the disease to susceptible animals.

These facts form a serious sequence of events, which constitute a danger not formerly appreciated in the administrative measures adopted to check the spread of the disease. What is the extent of the danger? A part of this large and important query can be answered if one can give an idea of the percentage of *Glossina palpalis* that become infected with the virus of Sleeping Sickness after they have fed on the infected antelope.

Throughout all these experiments only clean laboratory-bred flies were employed. The fact that there is no hereditary transmission of trypanosomes in *Glossina palpalis* is considered to have been so conclusively proved, that two of the members of the Commission have allowed several hundreds of clean laboratory-bred flies to bite them. Further, no evidence has ever been obtained by the Commission that these flies became infected with any flagellate by contact with other flies or fouled cages. Thus, any flagellates found in the laboratory-bred *Glossina palpalis* in these experiments must be considered to be derived from the infected antelope.

In some of the experiments the flies were fed, for varying periods, upon fowls. As will be shown in a further paper, the Commission found an avian trypanosome in some of the fowls obtained for experimental purposes. It would, therefore, be a fair criticism to state that a percentage of the flagellates found on dissection of the *Glossina* were avian in origin, were it not for the fact that negative experiments went to prove that this fowl trypanosome did not develop in the *Glossina*. It is also true that on one occasion the Commission thought they had succeeded in infecting a fowl with *Trypanosoma gambiense*; it may, therefore, be argued that the fowls fed upon in some of these experiments were naturally infected with *Trypanosoma gambiense*, and that the *Glossina* obtained their infection from such naturally-infected fowls and not from the antelope. Though many experiments were devised and carried out to try and confirm this one positive result, all efforts to infect fowls with Sleeping Sickness were so uniformly negative that the Commission must consider the one "positive" result to be an error.

A reference to Table 28 will show that *Glossina palpalis* were infected by antelope blood where no fowls were ever fed on; in fact, it will be noticed that a higher percentage of negative results were obtained where fowls were introduced into the experiments. In some of the experiments the flies were dissected as they died throughout the whole experiment, and in others the flies were dissected only when the experiment became positive. If the experiment was a negative one the flies were not dissected. In

order to avoid over estimations of the percentage of infected flies, no fly was called infected unless its gut was *swarming* with trypanosomes, and all the flies dissected during the earlier days of the experiments have been included. No fly was found to be infected with trypanosomes before the 19th day after its first infected feed on a buck. The tables otherwise explain themselves.

Table 28.—Giving the Percentage of Flies which became Infected when Fed on Infected Antelope. Flies fed at first on the infected antelope and afterwards only on healthy monkeys.

No. of experiment.	Species of antelope flies fed on.	No. of flies used.	No. of flies dissected.	No. of infected flies found.	Percentage of infected flies.	Remarks.
2346	Bush-buck 2328.	160	122	21	17.2	The gut-contents of 6 infected flies injected into a rat gave it Sleeping Sickness.
2384	"	100	91	10	11.0	No injection of infected flies.
2414	"	70	70	9	12.8	Five infected flies injected into a rat gave it Sleeping Sickness.
2501	"	100	57	12	21.0	No injection of infected flies.
2351	Reed-buck 2357.	100	84	9	10.7	" "
2507	Reed-buck 2359.	200	80	2	2.5	" "
2477	Bush-buck 2371.	60	47	4	8.5	" "
2478	Water-buck 2378.	60	—	—	—	Negative experiment. Flies not dissected.
2559	"	60	—	—	—	" "
2454	Reed-buck 2427.	110	92	13	14.2	One infected fly injected into a rat gave it Sleeping Sickness.
2485	Bush-buck 2428.	50	26	3	11.5	No injection of infected flies.
2460	Reed-buck 2429.	50	38	5	13.1	" "
2543	"	100	—	—	—	Negative experiment. Flies not dissected.
2544	Reed-buck 2431.	90	53	5	16.6	No injection of infected flies.
2592	"	100	78	1	1.3	" "
2476	Reed-buck 2445.	50	—	—	—	Negative experiment. Flies not dissected.

Remarks.—Of these 16 experiments, 12 were positive and 4 negative. In the positive experiments, 838 flies were dissected. Of these, 94 showed heavy intestinal infection with developmental forms of *Trypanosoma gambiense*, viz., 11.2 per cent. Of these infected flies, 43 were males and 51 females. If to these 838 flies

be added all the *Glossina palpalis* of the negative experiments, we get a total of 1,108 flies, and a percentage of 8·5 infected. In only 3 of the positive experiments was an injection made into susceptible animals of the citrated gut-contents of the infected flies. All three gave positive results.

The methods of procedure adopted in the experiments detailed in the following table were precisely the same as those of Table 28, with this one exception: The flies were, for varying periods, fed upon fowls as well as upon monkeys.

Table 29.—Giving the Percentage of Flies which became Infected when Fed on Infected Antelope. Flies fed at first on the infected antelope and afterwards on fowls, before being fed on healthy monkeys.

No. of experiment.	Species of antelope flies fed on.	No. of flies used.	No. of flies dissected.	No. of infected flies found.	Percent- age of infected flies.	Remarks.
2500	Reed-buck 2357.	100	—	—	—	Negative experiment. Flies not dissected.
2510	"	100	—	—	—	" "
2421	Bush-buck 2371.	50	—	—	—	" "
2499	Bush-buck 2372.	100	—	—	—	" "
2451	Water-buck 2378.	95	93	2	2·1	No injection of infected flies.
2456	Reed-buck 2427.	60	50	9	18·0	" "
2508	"	100	68	6	8·8	Three infected flies injected into a rat gave it Sleeping Sickness.
2464	Reed-buck 2431.	55	53	8	15·0	No injection of infected flies.

Remarks.—It will be seen that when the flies were fed on fowls and monkeys in these eight experiments, four were positive and four were negative. In the positive experiments 264 flies survived for dissection: of these 25 showed heavy intestinal infection with developmental forms of *Trypanosoma gambiense*, i.e., 9·47 per cent. Of the infected flies 11 were males and 14 females. If to these 264 flies be added all the *Glossina palpalis* of the four negative experiments, we get a total of 614 flies and a percentage of 4·0 infected. In the only experiment where an injection was made into a susceptible animal of the pooled citrated gut-contents of infected flies, the result was positive.

An analysis of Tables 28 and 29 brings out the following interesting points:—

In Experiment 2501, Table 28, it is seen that 21 per cent. of the *Glossina palpalis* were infected, out of the 57 flies that survived for dissection on the 47th day of the experiment. These

flies were infected by bush-buck, Experiment 2328, which had never shown *Trypanosoma gambiense* in its blood; and 55 days had elapsed since any infected *Glossina* had fed on this buck.

Sixteen out of the 24 experiments were positive. If all the *Glossina palpalis* dissected in these positive experiments be grouped together, it is seen that a total of 1,102 flies were examined. Of these, 119 flies—54 male and 65 female—were infected with developmental forms of *Trypanosoma gambiense*—that is, 10·8 flies in every hundred became infected, the sexes being about equally implicated. The highest percentage of infected flies in any one of the positive experiments was 21 per cent., in Experiment 2501, and the lowest was 1·3 per cent., in Experiment 2592. If to the total of 1,102 flies dissected in the positive experiments be added *all* the *Glossina* used in *all* the negative experiments, we get a total of 1,722 flies employed, and 6·9 flies in every hundred infected.

It is perhaps worth noting that a diet for the fly of antelope, fowl, and monkey blood gave a higher percentage of *negative* results and a lower percentage of flies infected than a diet of antelope and monkey blood only. This was quite unexpected, for the Commission, as a result of many experiments and considerable experience, were of the opinion that fowl's blood assisted the development of *Trypanosoma gambiense* in *Glossina palpalis*.

4. *How does Sleeping Sickness Affect the Health of Antelope?*

This point is of considerable importance. If the disease killed the antelope within a short time of infection, or even if it seriously affected their health so as to render them incapable or unwilling to move about freely, the facts detailed above would lose some part of their practical value.

The word "health" is not used here in a technical sense—that is to say, the health was not estimated by a series of blood counts and temperature charts. Interesting as such observations would have been, the Commission regret they were too short-handed and too much pressed by other work to carry them out.

Careful observations were made daily to answer the following questions:—Did the infected antelope during the time they were under observation appear sick? Did they become emaciated? Was there loss of health and strength? Were there corneal opacities, œdematous swellings, conjunctival discharges, or staring coats? These questions may at once be answered in the negative, except in the case of Reed-buck 2445, which will be referred to later below.

When the antelope were brought to the laboratory by the native hunters they invariably suffered from exhaustion, due probably to a combination of causes, such as fright, confinement for two to four days in cages too small to allow free movement, insufficient water and food, and the rough usage undergone when being caught. As a result of these unfavourable conditions, each antelope was kept under observation for a week or two before any experiments were undertaken. During this time they were

well fed and comfortably housed in reed kraals erected in a fly-proof house. Some of the antelope, especially the "oribi" and "entalaganya," died during the first fortnight. The 11 survivors were the subjects of these experiments.

Nine of these buck were kept under daily observation for four months after becoming infected with *Trypanosoma gambiense*. They remained apparently in perfect health.

The remaining two antelope were reed-buck. One, Experiment 2429, lived, and appeared very healthy, for 93 days after its infection. It then accidentally broke its leg and had to be killed. A *post-mortem* examination was made, and no evidence of trypanosomiasis was found. The other buck, Experiment 2445, arrived at the laboratory in a poor state of health, and died 12 days after its infection. There was no sign of trypanosomiasis at the *post-mortem* examination.

It is therefore evident that antelope infected with the virus of Sleeping Sickness may live in apparently perfect health for at least four months, and this, though they be kept under conditions less favourable than would occur in Nature, the constant handling and fly feedings to which these buck were subject being borne in mind.

5. Are Antelope Living in the Fly-Area Naturally Infected with Sleeping Sickness?

Positive evidence on the last query would complete the chain of evidence that antelope living in the fly-areas may act as a reservoir of the virus of Sleeping Sickness. So far it has only been proved that they are "potential" hosts.

The only method by which this query can be investigated is by capturing game in the fly-area and then—

1. Injecting its blood into animals susceptible to *Trypanosoma gambiense* infection.

2. Feeding cages of clean laboratory-bred *Glossina palpalis* on the newly-killed buck and subsequently endeavouring to infect animals susceptible to *Trypanosoma gambiense* with these flies. (The Commission know by observation that *Glossina palpalis* will feed readily on animals newly killed.)

Those who know the local conditions will appreciate the difficulty of carrying out these methods of investigation. It would be necessary for large drives of the buck to be organised in the fly-areas. Several hundred natives would be required. Large numbers of clean laboratory-bred *Glossina* must be available, many normal susceptible animals must be in readiness, and, at the same time, be so situated that they cannot be bitten by the possibly infected wild flies in the neighbourhood. The laboratory work in hand may have to be abandoned for the time being, and some 50 or 60 buck must be captured or killed before reliable information is forthcoming. The sun is hot, the country very difficult, and the exposure to the bites of the fly very great.

It may be said at once that the Commission were only able to shoot five buck on the Lake-shore. The blood of these gave negative results for *Trypanosoma gambiense* when injected into susceptible animals. One of them, however, gave a positive result for *Trypanosoma vivax*. This trypanosome was proved by the Commission to be not uncommonly carried by wild Lake-shore *Glossina palpalis*.^{*} It is therefore not unreasonable to suppose that at least one of the buck shot had been fed on by the Lake-shore fly.

It was recognised at length that occasional week-end shoots by a member of the Commission were quite inadequate. The assistance of the Acting-Governor of Uganda, S. C. Tomkins, Esq., C.M.G., the Acting Principal Medical Officer, Dr. C. Wiggins, of the Uganda Medical Staff, the Provincial Commissioners of Kampala, F. A. Knowles, Esq., C.M.G., and L. H. Cubitt, Esq., and of Sir Apolo Kagwa, K.C.M.G., the Prime Minister, was then sought to aid in organising a series of large drives of wild game in the fly-area in the neighbourhood of the laboratory. We are greatly indebted to these gentlemen for their ready efforts on our behalf, which resulted in a large drive being organised. Unfortunately, the Commission were ordered to leave the country before the drive could take place. The work on these lines, however, is being continued by Dr. R. van Someren, of the Uganda Medical Staff, and Captain A. D. Fraser, Royal Army Medical Corps, who were instructed to take over the laboratory work.

This very difficult question, therefore, still awaits its answer.

Conclusions.

1. Water-buck, bush-buck, and reed-buck can readily be infected with a *human strain* of the trypanosome of Sleeping Sickness by the bites of infected *Glossina palpalis*.

2. One exposure to the bites of infected flies is sufficient to infect an antelope with the virus of Sleeping Sickness.

3. Though the blood of an antelope may be proved to be infected with *Trypanosoma gambiense*, careful and continued examinations over prolonged periods may fail to reveal the presence of the parasite in the blood.

4. The incubation of the disease (Sleeping Sickness) in antelope is probably seven days.

5. Antelope of the water-buck, bush-buck, and reed-buck species, when infected with the virus of Sleeping Sickness, can transmit the infection to clean laboratory-bred *Glossina palpalis*.

6. This transmission of the infection to clean laboratory-bred flies may occur at least 81 days after the last feed of the infected flies on a buck.

7. *Glossina palpalis*, when infected with the virus of Sleeping Sickness obtained from the blood of infected antelope, are capable of transmitting the virus to susceptible animals.

^{*} *Vide* 'Proceedings' of the Royal Society, B, vol. 82, 1910, pp. 63—66; also Reports of the Sleeping Sickness Commission, No. X, p. 63.

8. An appreciable percentage of *Glossina palpalis* will become infected with the virus of Sleeping Sickness should these flies feed on antelope suffering from this disease.

9. It follows, from the above conclusions, that antelope living in the fly-areas are "potential" reservoirs of the virus of Sleeping Sickness.

10. No antelope up to the present has been found naturally infected with *Trypanosoma gambiense*.

11.—EXPERIMENTS TO ASCERTAIN IF THE UGANDA PIG (*POTAMOCHÆRUS CHÆROPOTAMUS*) MAY ACT AS A RESERVOIR OF THE VIRUS OF SLEEPING SICKNESS (*TRYPANOSOMA GAMBIENSE*).

Immense numbers of wild pig live in the forested and elephant-grass covered country of the Kingdom of Uganda and the Busoga Province. These animals lie up in the woods and grass during the day, and come out of their retreats between sunset and sunrise to feed on the sweet potato and other tubers which are extensively grown by the natives in their plantations. Formerly protected by Game Laws, these nightly marauders have latterly become so numerous, and inflict such damage on the crops, that orders are now issued by the Uganda Government for their wholesale destruction.

Amongst other game, these pig inhabit the depopulated two-mile area bordering the northern shores of Victoria Nyanza, and frequent the banks of fly-infested rivers and streams.

The Commission therefore endeavoured to procure live pigs for investigation, and to conduct experiments with them on lines similar to those carried out on antelope. Unfortunately, during the time that the wild-game reservoir question was being studied (March, 1910—August, 1910), the Commission only secured one pig. Full details of the experiment carried out on this pig are given below, and the result when compared with the results obtained from antelope is sufficiently interesting to warrant its publication.

The procedure was as follows:—A wild Uganda pig (*Potamo-chærus chæropotamus*) was caught in the Chagwe District of Uganda and brought alive to the laboratory. A preliminary inoculation of its blood was made into a normal white rat, to ascertain if the pig was infected with any trypanosome inoculable into a rat, e.g., *Trypanosoma gambiense*, *Trypanosoma brucei*, *Trypanosoma pecorum*, &c. This preliminary inoculation was made on June 13, 1910, and the result was negative, the rat being examined for one month after the injection.

Laboratory-bred and laboratory-infected *Glossina palpalis*, which were known to be infective, were then fed on the pig for six consecutive days (June 13-18, 1910). These flies were infected by a human strain of *Trypanosoma gambiense*.

Clean laboratory-bred *Glossina palpalis* were subsequently (June 20-27) fed on the pig, and to ascertain if they had become

infected by the pig's blood, they were fed for a further period on a normal monkey. This monkey remained healthy.

Given in tabular form, the various steps of this experiment are made clear.

Experiment 2561.

Trypanosoma gambiense "cycle," wild Uganda pig to monkey, by means of laboratory-bred *Glossina palpalis*.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. June 13 ...	—	Inoculated normal white rat with 5 minims of pig, Experiment 2556, blood.		Rat inoculated with pig's blood (preliminary inoculation) remained healthy.
" 13—18	1—5	Pig, Experiment 2556, fed upon by laboratory-infected <i>G. palpalis</i> .		These <i>G. palpalis</i> were known to be infective, and were infected by a human strain of <i>T. gambiense</i> .
" 20—27	7—14	Fed clean laboratory-bred <i>G. palpalis</i> on pig, Experiment 2556.		100 flies used.
28— Aug. 3	15—50	Flies which had fed on the possibly infected pig from June 20—27 were fed daily for 5 minutes on a normal monkey, Experiment 2581.	—	Monkey, Experiment 2581, was examined bi-weekly for <i>T. gambiense</i> from June 28 till Sept. 8, 1910; no trypanosomes ever seen. Monkey remained healthy. Flies which had fed on pig from June 20—27 had therefore not become infected.

Remarks.—One detail has been omitted from the above table. On June 20, seven days after the infected flies first feed on the pig, 1 cc. of the pig's blood was inoculated into a normal white rat, Experiment 2570, to ascertain if the pig's blood was capable of infecting the rat with *Trypanosoma gambiense* on inoculation. The rat remained healthy. It is thus seen, that though the pig had been fed on by infected flies for six days, its blood was subsequently not capable of infecting a susceptible animal on inoculation; nor did it infect the flies which fed upon it.

Conclusion.

No conclusion of any value can be drawn from a single experiment such as the above. It would appear, however, from this experiment, that the Uganda pig is not susceptible to *Trypanosoma gambiense* infection.

12.—EXPERIMENTS TO ASCERTAIN WHETHER THE BLOOD OF CROCODILES IS INFECTIVE AFTER THESE REPTILES HAVE BEEN INOCULATED WITH BLOOD CONTAINING *TRYPANOSOMA GAMBIENSE*.

Experiment 1793.

October 8, 1909.—Two crocodiles, a few days old, received 0.5 cc. each of blood from a monkey heavily infected with *Trypanosoma gambiense*. The blood of the crocodiles was proved to be free from trypanosomes before the experiment commenced.

October 20, 12 days later, some blood from the crocodiles was injected into monkey, Experiment 1846.

Monkey, Experiment 1846, was examined for one month.

Result.—Negative.

Experiment 1793 was repeated with two more young crocodiles, with negative results.

Conclusion.—From these experiments it would seem that *Trypanosoma gambiense* is incapable of living and multiplying in the blood of the crocodile.

13.—EXPERIMENTS TO ASCERTAIN IF THE DOMESTIC FOWL OF UGANDA MAY ACT AS A RESERVOIR OF THE VIRUS OF SLEEPING SICKNESS (*TRYPANOSOMA GAMBIENSE*).*

Birds of various species are very numerous on the shores of Lake Victoria. Of these, cormorants, darters, herons (African grey and purple, and other species), ibises (glossy and sacred), fish-eagles, weaver birds (various species), terns (various species), gulls, geese (Egyptian and spur-winged), plovers (various species), pratincoles, storks, kingfishers and gallinules are the most common. These birds all inhabit areas where the *Glossina palpalis* are numerous, and some evidence is forthcoming that in Nature the fly feeds on avian blood.†

In view of the continued infectivity of the fly in the depopulated areas of the Lake-shore, it is clear that every effort should be made to ascertain the source of such infectivity. Search for a reservoir of the virus of Sleeping Sickness at once suggests itself, and to those acquainted with the fauna of the Lake-shore, an avian host would be included amongst the various species requiring investigation.

Domestic fowls were employed in these experiments. They are readily obtained, easily handled, and the flies feed greedily on

* Reprinted from the 'Proceedings' of the Royal Society, B, vol. 83, 1911.

† 'Proceedings' of the Royal Society, B, vol. 82, 1910, p. 496, Table IV. ; and Table 42, p. 118, of this Report.

them. One species of trypanosome may in Nature only affect one species of avian host; therefore, it follows that whatever the result of this series of experiments may be, that result is only applicable to the fowl. The Commission trust that no wider significance will be attached to the conclusions set forth at the end of this paper: in other words, because a Uganda fowl is or is not a reservoir of *Trypanosoma gambiense*, it does not follow that a cormorant, or other bird, is or is not a reservoir. It would have been better for this reason to have experimented with one or other of the various species of wild Lake-shore birds. The difficulties were, however, too great at the time these experiments were carried out. It is hoped that this paper will stimulate rather than discourage other workers to carry on this line of investigation.

In order to discover whether Uganda fowls can act as a reservoir of the virus of Sleeping Sickness, the Commission asked themselves the following questions:—

1. Can fowls be infected with the virus of Sleeping Sickness by the bites of laboratory-bred and laboratory-infected *Glossina palpalis*?

2. Can fowls transmit the virus of Sleeping Sickness (*Trypanosoma gambiense*) to clean laboratory-bred *Glossina palpalis*? If this is possible, can the flies so infected convey the virus to normal susceptible animals?

An answer in the affirmative to those two questions would throw suspicion on a possible avian host. To decide these points, the following experiments were devised and carried out:—

Can Fowls be Infected with the Virus of Sleeping Sickness (Trypanosoma gambiense) by the Bites of Laboratory-bred and Laboratory-infected Glossina palpalis?

The method adopted to infect the fowls was carried out as follows:—Clean *Glossina palpalis*, hatched out in the laboratory, were given their first feeds on a monkey whose blood contained many *Trypanosoma gambiense*. An interval of at least 24 hours was then allowed to elapse before the infected flies were first fed on the fowl. The fowl was then fed on, for varying periods, by these flies. To make certain the flies were infective, their infectivity was usually tested by their being allowed to bite normal susceptible animals during the experiment. Finally, to discover if the fowl had become infected with *Trypanosoma gambiense* by the bites of the *Glossina palpalis*:—

(a.) The fowl's blood was examined frequently throughout the experiment for *Trypanosoma gambiense*.

(b.) The fowl was killed and its centrifuged heart or jugular vein blood was carefully examined microscopically for *Trypanosoma gambiense*.

(c.) Two or more cubic centimetres of the centrifuged blood were injected into a normal susceptible animal on one or more occasions during the experiment.

Two typical experiments are given in detail.

Experiment 1915a.

Date.	Day of experiment.	Precedure.	Result.	Remarks.
1909. Nov. 22—25	1—3	Flies fed on <i>T. gambiense</i> - infected monkey.		250 laboratory bred <i>G. palpalis</i> used.
„ 26— Dec. 31	4—39	Flies fed on Cock 1926.	—	<i>T. gambiense</i> infected flies found on Dec. 13, 14, 16, 17, 20, and 27.
1910. Jan. 1—10...	40—49	Flies fed on normal monkey, Experiment 1999, to test their infectivity.		100 flies of this experiment survived to 50th day and were used for Experiment 1915b.
„ 11 ...	50	Monkey, Experiment 1999, examined; blood showed <i>T. gambiense</i> .	+	Allowing 7 days for the incubation of <i>T. gambiense</i> in this monkey, it is seen that the flies infected it on Jan. 4—that is, 4 days after feeding on fowl.

Remarks.—On December 17, 1909, 1 c.c., and on December 22, 3 c.c. of the blood of cock, Experiment 1926, were injected into a normal monkey, Experiment 1954. This monkey remained healthy. On January 10, 1910, the cock was killed, 10 c.c. of its heart's blood were centrifuged, and the "buffy layer" carefully examined for *Trypanosoma gambiense*, with negative results. The whole of the 10 c.c. of blood obtained were then injected into two monkeys: 5 c.c. into monkey, Experiment 1954, and 5 c.c. into monkey, Experiment 2054. The blood of these two animals was examined frequently for a month after these injections. No trypanosomes were ever found and the monkeys remained healthy.

Experiment 2081.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. Jan. 9—14...	1—5	Flies fed on <i>T. gambiense</i> - infected monkey.		120 laboratory-bred <i>G. palpalis</i> used.
„ 15— Feb. 2	6—24	Flies fed on cock, Experiment 2074.	—	Feb. 15, monkey, Experiment 2135, showed <i>T. gambiense</i> in its blood.
Feb. 3—7 ...	25—29	Flies fed on normal monkey, Experiment 2135, to see if flies are infective.	+	Feb. 22, monkey, Experiment 2178, showed <i>T. gambiense</i> in its blood.
„ 8—14...	30—36	Flies again fed on cock, Experiment 2074.	—	Fowl, Experiment 2074, has therefore been fed on by infective <i>G. palpalis</i> .
„ 15—19...	37—41	Flies fed on normal monkey, Experiment 2178, to see if flies still infective.	+	

Remarks.—Cock, Experiment 2074, was killed on March 16, 1910; 10 c.c. of its heart's blood were taken and centrifuged and carefully examined for *Trypanosoma gambiense*, with negative results. The 10 c.c. were then injected, subcutaneously, into normal monkey, Experiment 2301. This monkey was examined bi-weekly up to April 15, 1910. No *Trypanosoma gambiense* were ever seen, and the monkey remained healthy.

The 13 experiments carried out on these lines are now given in tabular form, in Table 30 below:—

Table 30.

No. of experiment.	No. of flies used.	No. of fowl fed on.	No. of infected flies found during experiment.	Result.	Remarks.
1915a	250	Expt. 1926	7	Negative	Proved infective flies fed on this fowl.
1915b	100	" 2059	1	"	Proved infective flies fed on this fowl.
1915c	50	" 2182	—	"	Doubtful whether infected flies fed on this fowl.
1918	100	" 1927	—	"	Proved infective flies fed on this fowl.
2081	120	" 2074	2	"	Proved infective flies fed on this fowl.
2082	100	" 2087	12	"	Proved infective flies fed on this fowl.
2208	100	" 2209	0	"	Proved infective flies fed on fowl, but no flies dissected.
2325	160	" 2334	2	"	Proved infective flies fed on fowl.
2362	110	" 2363	7	"	Proved infective flies fed on fowl.
2365	300	" 2336	0	"	Flies not dissected, but were proved infective.
2366	150	" 2364	0	"	Flies not dissected, but were proved infective.
2390	180	" 2367	7	"	Proved infective flies fed on fowl.
2395	100	" 2396	1	"	Proved infective flies fed on fowl.

Remarks.—In these experiments it is seen that 1,820 laboratory-bred and laboratory-infected *Glossina palpalis* were fed on 13 fowls, with uniformly negative results. With one exception, Experiment 1915c, the flies were proved to be infective by feeding them on animals susceptible to *Trypanosoma gambiense* infection. That the fowls had not become infected with *Trypanosoma gambiense* was proved by carrying out the three tests given above, under headings a, b, and c. In Experiment 2325, the liver, spleen, and bone-marrow of Fowl 2334 were also examined for trypanosomes, and 10 c.c. of the pooled pulp injected into a normal monkey. This monkey remained healthy.

A further series of experiments bearing on this question was carried out. These experiments are not so complete. The procedure was as follows:—

A varying number of laboratory-bred *Glossina palpalis* were fed for several days on *Trypanosoma gambiense*-infected antelope. The infected flies were then fed for varying periods on fowls. The fowls were then killed and 2 c.c. of their blood were injected into a normal monkey. The monkey's blood was examined for *Trypanosoma gambiense* bi-weekly for a month after the injection of the fowl's blood. The experiments are given in tabular form:—

Table 31.

Date.	Days of "cycle" the flies fed on fowl.	Experiment No. of fowl.	No. of flies used.	Did flies subsequently prove to be infective?	Result of inoculation of fowl's blood into normal monkeys.
1910.					
May 9—21 ...	7—19th	Cock 2455	50	No	Negative
" 13—31 ...	4—22nd	" 2471	60	Yes (18 p. c.)	"
" 16—June 1	7—23rd	" 2479	95	" (2.1 p. c.)	"
" 16— " 1	4—20th	" 2480	55	" (15 p. c.)	"
June 2—11 ...	10—19th	" 2518	100	No	"
" 2—14 ...	5—17th	" 2519	100	"	"
" 2—17 ...	8—23rd	" 2519	100	Yes (8.8 p. c.)	"
" 2—11 ...	11—20th	" 2519	100	No	"

Remarks.—The chief criticism against these experiments is the fact that the flies fed on the fowls during the earlier days of the experiments, when the *Glossina palpalis* are usually not infective. However, as results have been recorded by the Commission where *Glossina palpalis* have infected susceptible animals as early as the 18th day of an experiment, it has been decided to publish these experiments.

Can Fowls Transmit the Virus of Sleeping Sickness to Clean Laboratory-bred Glossina palpalis? If this is possible, can the Flies so Infected convey the Virus to Normal Susceptible Animals?

In view of the results obtained by the first series of experiments, it would seem somewhat unnecessary to follow this question further.

It has so far been proved that the blood of fowls which have been fed upon by infected *Glossina palpalis* is non-infective when injected into susceptible animals. It has now to be ascertained if the blood of such fowls is also incapable of infecting the fly. This would constitute additional evidence against the fact that fowls act as a reservoir of *Trypanosoma gambiense*.

Cages of laboratory-bred and laboratory-infected *Glossina palpalis*, which were known to be infective, were fed on fowls for a varying number of days. Next, clean laboratory-bred flies were fed on these fowls for several days. The flies were then fed on normal susceptible animals (monkeys), in the endeavour to infect such animals with *Trypanosoma gambiense*, and so prove

that the blood of the fowl was infective. The full details of three experiments are given below:—

Experiment 2018.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. Jan. 4—10	1—6	Flies fed on Fowl 1927.		100 laboratory-bred <i>G. palpalis</i> used. Fowl, Experiment 1927, has been fed on for 29 days by <i>T. gambiense</i> -infected <i>G. palpalis</i> .
" 11 ... " 12— Feb. 23	7 8—50	Flies starved. Flies fed on Monkey 2061.	—	87 <i>G. palpalis</i> dissected throughout this experiment between the 11th and 55th days after flies' first feed on Fowl 1927. All were negative for flagellates. Monkey 2061 examined, with negative result, till March 7.

Experiment 2019.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. Jan. 4—10	1—6	Flies fed on Fowl 1926.		100 laboratory-bred <i>G. palpalis</i> used. Fowl, Experiment 1926, has been fed on for 29 days by <i>T. gambiense</i> -infected <i>G. palpalis</i> .
" 11 ... " 12— Feb. 23	7 8—50	Flies starved. Flies fed on Monkey 2062.	—	80 <i>G. palpalis</i> dissected throughout this experiment between the 11th and 55th days after flies' first feed on Fowl 1926. All were negative for flagellates. Monkey 2062 was examined bi-weekly for <i>T. gambiense</i> until March 22; no trypanosomes were seen. Monkey remained healthy.

Experiment 2276.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. March 7—12	1—5	Flies fed on Fowl 2087.		200 laboratory - bred flies used. Fowl, Experiment 2087, has been fed on for 18 days by <i>T. gambiense</i> -infected <i>G. palpalis</i> .
" 13 ... " 14— April 16	6 7—40	Flies starved. Flies fed on Monkey 2344.	—	116 <i>G. palpalis</i> dissected throughout this experiment, between the 23rd and 42nd days after flies' first feed on Fowl 2087. All were negative for flagellates. Monkey 2344 was examined bi-weekly for <i>T. gambiense</i> till May 10; no trypanosomes ever seen. Monkey died of broken neck later.

Remarks.—It will be seen that 400 laboratory-bred *Glossina palpalis* fed on the three fowls, Experiments 1927, 1926, and 2087, subsequently failed to infect monkeys with the virus of Sleeping Sickness, and that all the 283 *Glossina* dissected throughout these experiments were negative for flagellates. This absence of flagellates in the flies is interesting, because Fowl 1927 and Fowl 1926 were both found to be naturally infected with a large avian trypanosome. It would thus seem that this fowl trypanosome does not undergo development in *Glossina palpalis*.

Conclusion.

The Uganda fowl cannot act as a reservoir of the virus of Sleeping Sickness.

14.—EXPERIMENTS TO ASCERTAIN WHETHER THE MAMMALS, BIRDS OR REPTILES IN UGANDA, LIVING ON OR NEAR THE LAKE-SHORE, HARBOUR TRYPANOSOMA GAMBIENSE.

We have dealt elsewhere with the question of the continued infectivity of *Glossina palpalis* on the shores of Lake Victoria after the removal of the native population.* One of the possible explanations of this long duration of infectivity is the existence of some animal from which successive generations of *Glossina palpalis* are able to obtain the virus of Sleeping Sickness.

* 'Proceedings' of the Royal Society, B, vol. 82, 1909, p. 56; and Reports of the Sleeping Sickness Commission, Nos. X. and XI., pp. 56 and 58.

It has been shown that cattle may carry *Trypanosoma gambiense* in Nature,* and that antelope are at least potential hosts of the parasite.

The present paper represents an attempt to ascertain whether any other animal living on the Lake-shore acts in a similar way. It was necessary to find out, first, whether the men who caught the flies on the Lake-shore, or those who rowed them to and from their work, were infected with Sleeping Sickness, and the first experiments show the results of the inoculation and examination of the blood of these men.

Inoculation and Examination of the Blood of Human Beings.

Experiment 1224.

June 28, 1909. The blood of canoe-men and fly-boys, fourteen in all, who had been employed for the previous six months on the Lake-shore, was examined. One cubic-centimetre of the blood of each was pooled and injected into a monkey. This animal was examined for forty-three days, but it did not develop Sleeping Sickness.

Result.—Negative.

Experiment 1224 was repeated on September 30 and November 30, 1909, with a negative result.

Experiment 2121.

January 31, 1910. $\frac{1}{2}$ c.c. of the blood of each of the six fly-boys and thirteen canoe-men, employed on the Lake-shore, was pooled and injected into two monkeys. The examination of the monkey was continued for a month.

Result.—Negative.

A procedure similar to Experiment 2121 was adopted with regard to the inoculation of the blood of the fly-boys and canoe-men, employed on the Lake-shore, during February, March, April, May and June, 1910, but the result was negative in every instance.

Frequent microscopical examination of the blood of the fly-boys and canoe-men failed to reveal the presence of trypanosomes. The parasites of malaria and filaria were frequently detected in the blood.

A constant watch was kept for any symptoms of Sleeping Sickness appearing in these fly-boys and canoe-men, but none was noted.

The same fly-boys remained with the Commission throughout its enquiry in Uganda, viz., from October, 1908, to August, 1910, but the canoe-men were substituted for others once or twice during that period.

Examination of the Blood of Monkeys.

Seven monkeys from the island of Kome, two from the island of Buvuma, and two from a promontory at Jinja, were all examined,

* 'Proceedings' of the Royal Society, B, vol. 82, 1910, p. 480; and p. 71 of this Report.

with negative results. All three of these localities are heavily infected with Sleeping Sickness. Sixteen other monkeys, obtained from various parts of the Lake-shore, were also examined, but no trypanosomes were found.

In addition to these experiments, it may be pointed out that every monkey used by the Commission was examined before it was used for any experiment. It is estimated that considerably more than one thousand monkeys were utilised during the Commission's stay in Uganda. Control monkeys were always kept along with the experimental animals, and none of the former ever showed signs of Sleeping Sickness.

None of these monkeys, except one, from among the large number examined by the Commission, ever showed trypanosomes, except as the result of an experiment. The locality from which the one naturally-infected monkey was obtained was not definitely ascertained, but it was of the species *Cercopithecus pygerythrus centralis* which inhabit the forests around the Lake-shore. This is a grey monkey with a black face and is one of the common monkeys in Uganda.

It is difficult to explain why more naturally-infected monkeys were not found, because many of these animals were caught in fly-areas, and the animals, though free from Sleeping Sickness in their wild state, showed themselves readily susceptible to artificial infection.

Table 32.—Inoculation of the Blood of other Mammals.

Experiment No.	Species of animal.	Locality in which caught or shot.	Animal injected.	Amount of blood.	Time after death.	Result.
531	Cobus antelope.	Inland	Rat	?	Few minutes	Negative.
589	"	"	"	?	"	"
591	"	"	"	?	"	"
607	"	"	"	?	"	"
1526	"	"	Goat	?	One hour	"
1530	"	"	"	?	Few minutes	"
1531	"	"	Rat	?	"	"
1534	" (3)	"	Goat	?	1½ hours	"
1535	" (3)	"	Rat	?	Few minutes	"
1540	" (3)	"	Goat	?	Two hours	"
1541	" (3)	"	Rat	?	"	"
532	Water-buck	"	"	?	Few minutes	"
1524	" (2)	"	Goat	?	One hour	"
1536	"	"	"	?	Few minutes	"
1537	"	"	Rat	?	"	"
1538	"	"	Goat	?	"	"
1539	"	"	Rat	?	"	"
2378	"	"	Monkey	5 c.c.	Alive	"
606	Reed-buck	"	Rat	?	Few minutes	"
2357	"	"	Monkey	5 c.c.	Alive	"
2359	"	"	"	5 c.c.	"	"
2369	"	"	"	5 c.c.	"	"
2415	"	"	"	5 c.c.	Few minutes	"
2427	"	"	Rat	1 c.c.	Alive	"
2429	"	"	"	5 c.c.	"	"

Table 32.—Inoculation of the Blood of other Mammals—
continued.

Experiment No.	Species of animal.	Locality in which caught or shot.	Animal injected.	Amount of blood.	Time after death.	Result.
2429	Reed-buck	Inland	Rat	1 c.c.	Alive	Negative.
2431	"	"	"	1 c.c.	"	"
618	Bush-buck	?	Monkey	5 c.c.	Few minutes	"
648	"	?	"	2 c.c.	"	"
1920	"	Lake-shore	"	5 c.c.	"	"
1921	"	"	"	5 c.c.	"	"
1922	"	"	Goat	10 c.c.	"	<i>T. vivax</i> appeared 16 days later.
2291	"	"	Monkey	5 c.c.	"	Negative.
2292	"	"	Goat	10 c.c.	"	"
2371	"	Inland	Monkey	5 c.c.	Alive	"
2372	"	"	"	2 c.c.	"	"
2373	"	"	"	2 c.c.	"	"
2374	"	"	"	2 c.c.	"	"
2428	"	"	Rat	1 c.c.	"	"
2424	" Oribi "	"	Monkey	5 c.c.	"	"
2425	"	"	"	5 c.c.	"	"
2521	"	"	Rat	2 c.c.	"	"
2522	"	"	"	2 c.c.	"	"
2602	Elephant	"	Guinea-pig.	2.5 c.c.	Few minutes	"
2603	"	"	"	2.5 c.c.	"	"
592	Zebra	"	Rat	?	"	"
1527	"	"	Goat	?	"	"
1532	"	"	"	?	"	"
1533	"	"	Rat	?	"	"
2237	Wild rat	"	"	1 c.c.	"	"

Remarks.—It will be seen from the above table that five animals only were shot on the Lake-shore, one of which, a bush-buck, was found to harbour *Trypanosoma vivax* in its blood. The remaining animals, all from inland localities, proved negative.

Table 33.—Inoculation of the Blood of the Crocodile and Hippopotamus.

Experiment No.	Species of amphibian.	Locality in which shot.	Animal injected.	Amount of blood.	Time after death.	Result.
1568	Crocodile	Lake-shore	Monkey	2 c.c.	2½ hours	Negative
608	Hippopotamus	"	"	10 c.c.	18 "	"
1674	"	"	"	7 c.c.	—	"
1697	"	"	"	8 c.c.	15 hours	"
1762a	}	"	"	10 c.c.	5 "	}
1762b				pooled.	6 "	
1851				12 c.c.	6 "	
2337	"	"	"	10 c.c.	6 "	"

Result.—Negative.

Table 34.—Inoculation of the Blood of Birds.

Experiment No.	Species of bird.	Locality in which shot.	Animal injected.	Amount of blood.	Time after death.	Result.
1351	Eight divers	Lake-shore	Monkey	2 c.c. of each.	At once	Negative
1352	One jacana One fishing eagle.	}	"	"	"	"
	Two gulls. One plover					
1353	Two cormorants	"	"	"	"	"
1568	One jacana. Four darters	}	"	"	"	"
1804	Two Egyptian geese. One bronze stork.					
	Two paddy birds. Four divers	}	"	Pooled	"	"
1830	Six divers					
1874	Two darters	"	"	"	"	"

Result.—Negative.

In addition to these blood-injection experiments, the microscopical examination of a large number of mammals, birds, &c., was carried out. These were shot in localities on the Lake-shore and inland.

Examination of the Blood of Mammals.

The mammals examined, 29 specimens in all, included bats, servaline cat, rats, squirrels, cobus antelope, water-buck, bush-buck, "oribi," and wild pig, and these all proved negative for *Trypanosoma gambiense*.

Examination of the Blood of Birds.

The birds examined included 138 representatives of the following species:—Guinea-fowls, plovers, hawks, buzzards, fish-eagles, vultures, divers, cormorants, ibises, geese, herons, francolins, kingfishers, bee-eaters, plantain-eaters, hornbills, doves, pigeons, wag-tails, coucals, green parrots, swallows, sparrows, rollers, gulls, jacanas, pratincoles, storks, egrets, quail and bustard. A large number of these showed various species of blood parasites which form the subject of another paper. None contained *Trypanosoma gambiense*.

Examination of the Blood of Reptiles, &c.

The blood of 31 specimens of crocodiles, iguanas, frogs and tortoises, was also examined, and the result was also negative for *Trypanosoma gambiense*.

Conclusions.

1. With the exception of one monkey, no evidence was obtained that the Lake-shore and other wild animals, birds and reptiles, harbour the virus of Sleeping Sickness.

2. A bush-buck shot on the Lake-shore was found to harbour *Trypanosoma vivax* in its blood.

15.—EXPERIMENTS TO INVESTIGATE THE INFECTIVITY OF *GLOSSINA PALPALIS* FED ON SLEEPING SICKNESS PATIENTS UNDER TREATMENT.*

Introduction.

It is well known that there are cases of Sleeping Sickness in man which, under treatment, may enjoy long periods of apparent good health. In these cases the individual is often able to live an active life, and insists on being at liberty to go about as he pleases. In Uganda care is taken to prevent such cases entering the fly-areas; but the difficulty in maintaining a supervision strict enough to check occasional visits to these areas is obviously great, and in other countries may be insuperable, so that the question as to how drug treatment can influence the infectivity of these patients to the fly may be considered to be of importance.

Other points arise from the consideration of this question, viz.:—Can one or more doses of a trypanocidal drug render a Sleeping Sickness patient non-effective to *Glossina palpalis*? Does prolonged treatment during any stage of the disease render cases of Sleeping Sickness innocuous to the fly? If it be proved that the treatment of Sleeping Sickness patients by certain drugs does not prevent them infecting *Glossina palpalis*, then what percentage of cases give a positive result? and what percentage of flies are infected from these cases, as compared with the number of flies infected from untreated cases?

To answer these questions it is necessary to classify under various headings the patients who are the subjects of this paper; to state whether treated or untreated; if the former, to give details concerning the nature of the treatment, the duration of treatment, and the total quantity of drugs received. The presence or absence of *Trypanosoma gambiense* in the blood or lymph glands of each patient should also be noted.

The classification of patients, according to the stage of the disease in which they present themselves, is that adopted by Dr. A. D. P. Hodges, C.M.G., Principal Medical Officer, Uganda, in the "Progress Report on the Uganda Sleeping Sickness Camps," from December, 1906, to November 30, 1908.

Class A.—Those who are apparently in good health: gland enlargement with trypanosomes present in the glands.

Class B.—Those in whom there are early signs from which the existence of Sleeping Sickness may be reasonably suspected.

Class C.†—Those in whom clinical signs are well marked.

Class D.†—Advanced cases.

These experiments were carried out from January to March, 1910. Of the 33 patients under observation,

6	belonged to Class A.
19	" " B.
7	" " C.
1	" " D.

The method adopted to test the degree of infectivity of patients to the fly in different stages of the disease were: (1) By mechanical transmission from Sleeping Sickness patient to normal monkey,

* Reprinted from the 'Proceedings' of the Royal Society, B, vol. 83, 1911.

† Classes C and D are not now differentiated.

and (2) by transmission of the disease from Sleeping Sickness patient to normal monkey after the parasite had completed its development in the fly.

1. *By Mechanical Transmission from Sleeping Sickness Patient to Normal Monkey.*

The procedure in testing the first method was as follows:—A cage of laboratory-bred flies was first placed for two minutes upon the bare shoulders of a patient suffering from Sleeping Sickness, which was time enough to allow the majority of the flies to drawn some blood, but not enough to satisfy them. After an interval of five minutes the cage was transferred to the abdomen of a normal monkey, where it remained for two minutes, by which time nearly all the flies had completed their feed. This procedure was repeated daily for six days.

Ten of the patients under experiment had been treated with soamin and perchloride of mercury administered by subcutaneous injection. Two patients, who had been under treatment for one year, had received several doses of atoxyl, in addition to soamin and mercury.

Trypanosomes had been found at one time in the glands of all the patients, but no trypanosomes were found in their blood during the time the flies were being fed upon them.

Table 35.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients to Healthy Monkeys by Interrupted Feeding.

No. of experiment.	Class of patient.	If treated.	Duration of treatment.	No. of doses.	No. of flies fed.	No. of times flies fed.	Result.
2014	A	Yes	7 days	2	75	6	—
2137	B	"	7 "	2	65	7	—
2144	C	"	7 "	2	75	6	—
2146	B	"	7 "	2	65	6	—
2013	A	"	30 "	4	75	6	—
2027	B	"	30 "	4	75	6	—
2029	B	"	30 "	4	75	6	—
2150	C	"	30 "	4	70	7	—
2152	C	"	30 "	4	50	7	—
2158	B	"	30 "	4	60	6	—
2139	B	"	1 year	?	70	6	—
2154	B	"	1 "	?	50	6	—
2012	B	No	Nil	Nil	75	6	—
2281	B	"	"	"	100	6	—
2284	B	"	"	"	50	6	—

Remarks.—These experiments are all negative—a not unexpected result now that it is known that the mechanical transmission of Sleeping Sickness may be a somewhat rare event in Nature. The question as to whether the medicinal treatment of Sleeping Sickness patients has any preventive influence on this method of transmission remains unanswered. It may, however,

be noted that in the two untreated cases, viz., Experiment 2281, Class B, and Experiment 2284, Class B, the disease was not transmitted mechanically, but, as will be seen later (Table 37, Experiments 2280 and 2298), was conveyed after a period of development of the parasite within the fly.

2. *By Transmission of the Disease from Sleeping Sickness Patient to Normal Monkey after the Parasite had Completed its Development in the Fly.*

The procedure adopted in the second method for transmitting the disease from a Sleeping Sickness patient to a monkey was as follows:—A cage of laboratory-bred flies was fed daily for five or six days upon a patient suffering from Sleeping Sickness. They were then starved for 48 hours, and subsequently fed daily for 50 days upon a normal monkey.

In most of these experiments the flies were dissected and examined for flagellates after the period of feeding upon the normal monkey. The monkey's blood was subsequently examined twice weekly for one month after the last date on which the flies were fed upon it.

Trypanosomes had been found at one time in the glands of all these patients, but no trypanosomes were found in their blood during the time the flies were being fed upon them.

In the following tables all the details bearing on each class of patient are given:—

Table 36.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class A, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
1941	7 days	2	125	6	50	—	Monkey remained healthy.
1973	7 "	2	100	6	50	—	"
1969	1 month	4	100	7	50	—	"
1995	1 "	4	130	4	50	—	"
2240	2½ years	?	75	4	50	—	"
2252	2½ "	?	80	4	50	—	"

Remarks.—Reference to Table 36 will show that six patients in Class A stage of the disease were under treatment. Two of these had been under treatment for one week, and had each received two injections of soamin, 0·01 gramme, combined with perchloride of mercury, 0·02 gramme. Two had been under treatment for one month, and had received four injections of soamin, 0·01 gramme, and perchloride of mercury, 0·02 gramme, per dose. Two cases had been under treatment for two and a-half years: the total quantity of drugs received being, in one case, atoxyl, 16·2 grammes, perchloride of mercury, 2·4 grammes, and orpiment,

8·6 grammes; the other case had received 18·8 grammes of atoxyl, 2·4 grammes of perchloride of mercury, and 8·6 grammes of orpiment.

Sleeping Sickness was not transmitted from any of these patients to monkeys by the flies.

Two hundred and seventy-three flies were dissected between the 4th and 51st days after feeding upon infected patients, but no flies were found infected with flagellates.

Table 37.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class B, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
1993	7 days	2	100	4	50	—	Monkey remained healthy.
2091	7 "	2	50	5	50	—	"
2094	7 "	2	60	5	50	—	"
2170	7 "	2	60	6	50	—	"
1975	30 "	4	100	5	50	—	"
1987	30 "	4	65	5	50	—	"
1991	30 "	4	90	4	50	—	"
2068	30 "	4	65	6	50	—	"
2070	30 "	4	80	6	50	—	"
2107	30 "	4	65	6	50	—	"
2195	30 "	4	50	6	50	—	"
2248	30 "	4	65	4	50	—	"
2096	1 year	8	60	5	50	—	"
2109	1 "	9	65	5	50	—	"
2172	1 "	8	60	5	50	—	"
2193	1 "	9	60	6	50	—	"
2254	2½ years	?	80	4	50	—	"
2256	2½ "	?	60	4	50	—	"
2258	3½ "	?	60	4	50	—	"
1943	Nil.	Nil	65	6	50	—	"
1957	"	"	100	4	50	—	"
1967	"	"	110	6	50	—	"
2048	"	"	50	6	50	—	"
2280	"	"	130	4	50	+	<i>T. gambiense</i> appeared in blood.
2298	"	"	80	3	50	+	"

Remarks.—In Table 37, Class B, 25 experiments from 19 different patients are recorded. Six of these were from untreated cases.

Sleeping Sickness was transferred from two untreated patients to normal monkeys. The flies belonging to these experiments were not dissected.

The remaining four experiments from untreated cases were negative, both as regards infection of the flies (190 dissected) and the conveyance of the disease to monkeys.

Of the 19 negative experiments from cases under treatment, four were from two patients who had been under treatment for

one week. They had each received two injections of soamin in 1-gramme doses, combined with 0.02 gramme of perchloride of mercury. Eight experiments were from patients who had been one month under treatment. They had each received four injections (soamin 1 gramme, and perchloride of mercury 0.02 gramme, per dose). Four experiments were from two cases who had been under treatment for 11 months. One of these had received injections amounting in all to soamin 8 grammes, HgCl_2 0.10 gramme, and orpiment 2 grammes. The other patient had received a total amount of soamin 9 grammes, HgCl_2 0.10 gramme, and orpiment(?). Two experiments were from patients who had been under treatment for two and a-quarter years. They had each received a total quantity of drugs amounting to atoxyl 19.7 grammes, HgCl_2 0.06 gramme, orpiment 4.60 grammes, and soamin 4 grammes. One experiment was from a patient who had been three and a-half years under treatment, and had received during the last 18 months atoxyl 9.5 grammes, soamin 7.5 grammes, and orpiment 4.6 grammes.

From these 19 negative experiments 509 flies were dissected between the 4th and 52nd days after their last infected feed. No flagellates were found in any of them.

Table 38.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class C, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
2103	7 days	2	60	6	50	—	Monkey remained healthy.
2168	7 "	2	63	6	50	—	"
2250	7 "	1	100	4	50	—	"
2034	30 "	4	100	3	50	+	<i>T. gambiense</i> appeared in blood.
2098	30 "	4	50	5	50	—	Monkey remained healthy.
2105	30 "	4	65	4	50	—	"
2189	30 "	4	60	7	50	—	"
2191	30 "	4	65	7	50	—	"
2244	2½ years	?	120	4	50	—	"
2242	Nil	Nil	85	4	50	—	"

Remarks.—In Table 38, Class C, 10 experiments are recorded. One of these was from an untreated case with large numbers of trypanosomes in the cervical glands, but none detectable in the blood.

The monkey was not infected by flies fed upon this patient, and so the flies were not dissected.

Of the 9 experiments from treated cases, Class C, three were from two patients who had been under treatment for one week, of whom one had been given a single dose of soamin 2 grammes, and HgCl_2 0.04 gramme. The other patient had received two doses.

The experiments performed from these patients were negative as regards infection of both monkeys and flies.

Three patients had been under treatment for one month. Each of these patients had been given injections of soamin and perchloride of mercury, amounting to 2 grammes of the former drug and 0.08 gramme of the latter. One of these experiments was positive, both as regards infecting the flies and transmitting the disease to monkeys.

Seventy-five flies were dissected from the positive experiment, 2034. Of these, four flies were found to be infected with *Trypanosoma gambiense* on the 28th, 39th, 40th, and 46th day respectively after their last infected feed. The four flies found to be infected with trypanosomes were injected into a susceptible animal, which subsequently developed Sleeping Sickness.

Only one patient belonging to Class D. was available for these experiments. This patient had been under treatment for one and a-half years, and had been treated with a total quantity of 13.4 grammes atoxyl, 0.4 gramme perchloride of mercury, and 6.5 grammes orpiment.

Flies were not infected from this case, and the disease was therefore not transmitted to a normal monkey.

It is obvious that the results obtained from these experiments, though giving us some information, are insufficient to warrant a decisive answer to the questions before us.

With regard to the first query: Can treatment of Sleeping Sickness by the drugs named influence the infectivity of the fly? Omitting the mechanical transmission experiments, we may say that out of the 35 experiments only one produced a positive result, *i.e.* flies were infected and the disease transmitted from a Sleeping Sickness patient under treatment to a normal monkey. The case referred to above will also entitle us to express an opinion as to whether one or more doses of a certain trypanocidal drug can render a patient non-infective to the fly. It will be seen that this case had been under treatment for one month, and had been injected with four doses of trypanocidal drugs. The tsetse flies were infected 25 days after the last of the four injections had been administered.

Therefore, it may be said that to treat Sleeping Sickness patients with injections of soamin and perchloride of mercury, as specified, does not necessarily cause their blood to be incapable of infecting *Glossina palpalis* with *Trypanosoma gambiense*, and that flies so infected can transmit the disease to monkeys.

The percentage of flies infected from untreated cases was not ascertained, as only 190 flies were dissected, at various intervals, after feeding on their patients, and none were found infected.

A total of 857 flies were dissected, at different periods, after having fed upon treated cases, and four of these were found to be infected, *i.e.* a percentage of 0.46. These four flies all came from a cage containing 100 *Glossina palpalis* which had been fed on three occasions upon the only treated case that proved infective to

the flies, *vide* Table 38, Experiment 2034. This patient therefore infected 4 per cent. of flies—an interesting and important fact.

It was hoped that patients under the influence of trypanocidal drugs would be incapable of infecting tsetse flies. Unfortunately, this experiment (2034) is sufficient to show that Sleeping Sickness patients under treatment with arsenical compounds, etc., are not always innocuous to the fly, and should not be allowed their freedom to wander about the country during intervals of comparative good health, but must be kept completely and permanently isolated from all possible contact with the fly.

Conclusions.

1. *Glossina palpalis* fed on natives suffering from Sleeping Sickness, and untreated by drugs, may become infected and be capable of transferring the disease to healthy animals.

2. *Glossina palpalis* fed on natives suffering from Sleeping Sickness, and treated by arsenic and other drugs, may also become infected and be capable of transferring the disease to healthy animals.

C. MISCELLANEOUS EXPERIMENTS.

16.—THE NATURAL FOOD OF *GLOSSINA PALPALIS*.*

As a good deal of interest, and it may be importance, attaches to the food of the tsetse fly—the carrier of Sleeping Sickness—the following notes are placed on record:—

In the laboratory it was found that the flies fed with far more avidity on birds than on monkeys, while they could hardly be tempted to feed on young crocodiles, iguanas, or lizards. It was very marked, this preference of theirs for birds; the moment a chicken was placed against the netting of the cage, they instantly swarmed on it in hundreds. From this it was thought probable that the natural food of the flies would prove to be birds' blood, but the two following experiments do not bear this out to any great extent:—

The first experiment was carried out in the laboratory at Mpumu, and extended over several months. Flies which had been caught on the Lake-shore and which had been kept over from the previous day, and sometimes longer, were dissected, and each portion of the alimentary canal examined in the fresh state under a low power. The various organs of the fly were then smeared separately, and after staining (with one of the modifications of the Romanowsky stain) were examined in the usual way.

The dissections were carried out as follows:—The pharynx and proboscis were removed with a needle, and having been placed under a cover-glass and slightly opened out by pressure were examined in the fresh state. The tube of the proboscis was in this way admirably displayed, and the presence of red blood corpuscles or of flagellates could readily be determined. The alimentary canal was then removed from the abdomen, and the salivary glands detached from the fat-body. The alimentary canal was then unravelled and laid out in its full length on a slide. The whole of the tube could then be passed in review, from the junction of the thoracic intestine with the fore-gut down to the rectum, and the contents at various levels determined, as far as possible, with medium-power magnifications. The main portions of the alimentary canal were then noted, and these were separated from one another by cross cuts with a sharp-edged dissecting needle. In this way the fore-gut, mid-gut, hind-gut, and proctodæum were separately removed and smeared on a slide. The smears (whilst still moist) were exposed to the vapour of osmic acid for a few seconds, and then passed through alcohol before washing and staining. The proventriculus can, with a little practice, be removed intact from the ventral aspect of the thorax. If necessary, the thoracic portions of the salivary ducts can also be recovered.

* Reprinted from the 'Proceedings' of the Royal Society, B, vol. 82, 1910.

Speaking in general terms, the contents of the various portions of the alimentary canal could be determined with considerable accuracy from the naked-eye appearance during dissection. If the fly has fed very recently, the blood oozes from its proboscis when it is handled. The proventriculus, the crop or sucking stomach, as well as the fore-gut, are greatly distended with red blood. When the fed fly is put aside the blood-cells generally disappear from the proboscis in a few hours, though they may exceptionally be found as long as 24 hours after a feed. Similarly, the proventriculus and thoracic intestine quickly empty themselves, and the crop discharges its contents into the upper alimentary canal within 24 hours.

The blood travels slowly down the alimentary canal, changing in appearance as it goes. The outline of the red cells can sometimes be distinguished three days after a feed, but only when they have been retained in the fore-gut. When the blood reaches the mid-gut it begins to disintegrate and becomes homogeneous and somewhat translucent; when it reaches the lower part of the mid-gut it first becomes dark and eventually black and tarry. When in this state all cell elements are lost, and the colouring matter of the red cells is recognisable in the form of amorphous black granules. When the contents pass posterior to the Malpighian tubes, that is, into the hind-gut, they at once become faecal in character, and turn into a dirty, yellowish-brown material, which is microscopically composed of fine granules. It is passed from the fly in this form.

The figures given in the subjoined table refer to flies which were caught at various parts of the Lake-shore, and were generally kept for about 24 hours before dissection. The majority of the dissections were done during October, November, and December, 1908.

Table 39.—The contents of the Alimentary Canal of 220 *Glossina palpalis*.

Total flies examined.	Intestine empty.	Intestine contained blood.	Character of Blood.		
			Mammalian.	Non-mammalian.	Non-recognisable.
220	160	60	20	9	31
Percentages	72·7	27·3	9·1	4·1	14·1
				27·3	

Remarks.—In two cases blood corpuscles were recognised as being derived from monkeys, as the characteristic parasites of monkey malaria were found in them.

The second experiment to ascertain the food supply of *Glossina palpalis* was modified as follows:—

A journey was made to a small peninsula, hereafter referred to as "Crocodile Point." This place was distant about two and a-half to three hours by canoe from the Kibanga landing-place, and lay in a sheltered bay far out of the beaten track of the canoes which come backwards and forwards between the islands of Kome, Damba, and Buvuma, and the weekly market at Kibanga clearing. It was chosen, therefore, partly because it was isolated from human influences, and also because of the large number of flies which lived there and the number of crocodiles and birds which frequented it.

When it was first visited, a large crocodile was disturbed from where she was lying outstretched on a spit of sand. The canoe-men at once set to work, and unearthed 58 crocodile eggs lying in layers about 18 inches below the surface, over which the form of the parent was clearly defined in the soil.

The peninsula was pointed in shape, and not more than 60 yards long, and was closed on the land side by the dense wall of forest which fringes the Lake-shore. It was scattered with light undergrowth and fringed by ambatch trees, on which flocks of divers and cormorants sat with outstretched wings drying in the sun. A barrier of bare rocks and boulders projected on one side, and a small school of hippopotami was generally to be seen floating near and basking in the sun. Many small crocodiles were disturbed from the undergrowth as the point was explored, and various kinds of small land birds frequented the reeds and shrubs. The soil was sandy loam, and shaded by the light foliage. *Glossina palpalis* swarmed. The place has been described at some length, so that the exact conditions may be realised.

Subsequent to this experiment, the daily catch of Lake-shore flies was obtained from this place, and incidentally it may be added that the flies caught here were regularly found to be infective to monkeys.

Table 40.—The Naked-Eye Appearances of the Contents of the Alimentary Canal of 183 Tsetse Flies, with their Sex, and the Presence or Absence of a Larva.

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
1	M.	—	—	—	10	F.	—	—	—
2	M.	—	—	—	11	F.	—	—	—
3	M.	—	—	—	12	F.	—	—	—
4	F.	+	—	—	13	F.	—	—	—
5	F.	—	—	—	14	M.	—	—	—
6	M.	—	—	—	15	M.	—	—	—
7	M.	—	—	—	16	M.	—	—	—
8	F.	+	—	—	17	F.	—	—	+
9	M.	—	—	—	18	F.	—	—	+

Table 40.—The Naked-eye Appearances of the Contents of the Alimentary Canal of 183 Tsetse Flies, with their Sex, and the Presence or Absence of a Larva—*continued*

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
19	M.	—	+	—	71	F.	—	+	—
20	F.	—	—	+	72	M.	—	—	+
21	M.	—	+	—	73	F.	—	—	—
22	M.	—	—	+	74	F.	+	+	+
23	F.	—	—	+	75	M.	—	+	+
24	F.	—	—	+	76	M.	—	—	+
25	M.	—	+	—	77	M.	—	+	+
26	M.	—	—	+	78	F.	—	+	—
27	M.	—	+	+	79	M.	—	—	—
28	F.	+	—	+	80	M.	—	—	—
29	M.	—	—	+	81	F.	—	—	—
30	M.	—	—	—	82	M.	—	+	+
31	F.	—	—	+	83	F.	+	—	—
32	F.	+	+	—	84	F.	+	—	—
33	F.	—	—	+	85	M.	—	—	—
34	M.	—	—	—	86	F.	—	—	+
35	F.	—	+	—	87	M.	—	—	—
36	F.	—	—	+	88	F.	—	—	—
37	F.	+	—	—	89	M.	—	—	—
38	M.	—	+	—	90	M.	—	—	+
39	F.	—	—	+	91	F.	+	+	+
40	F.	—	—	+	92	F.	+	—	—
41	M.	—	—	+	93	M.	—	—	—
42	M.	—	+	—	94	M.	—	—	+
43	M.	—	—	+	95	M.	—	—	+
44	F.	+	—	—	96	M.	—	—	+
45	F.	—	—	+	97	M.	—	+	+
46	M.	—	—	+	98	F.	—	+	+
47	F.	—	—	+	99	M.	—	—	—
48	F.	—	—	+	100	M.	—	—	+
49	M.	—	—	+	101	F.	+	—	+
50	M.	—	—	—	102	F.	+	—	+
51	M.	—	—	+	103	M.	—	—	—
52	F.	+	—	—	104	F.	+	—	—
53	M.	—	—	—	105	M.	—	—	—
54	F.	—	+	+	106	F.	+	—	+
55	M.	—	—	—	107	M.	—	—	—
56	F.	—	—	+	108	M.	—	—	+
57	M.	—	—	—	109	M.	—	—	—
58	F.	—	—	—	110	M.	—	—	—
59	F.	+	—	+	111	F.	+	—	—
60	F.	—	—	+	112	M.	—	—	+
61	M.	—	—	+	113	F.	—	—	+
62	M.	—	—	+	114	M.	—	—	+
63	M.	—	—	+	115	M.	—	—	—
64	F.	—	—	—	116	M.	—	+	+
65	M.	—	—	—	117	F.	—	—	+
66	M.	—	—	+	118	M.	—	—	—
67	M.	—	—	—	119	F.	+	—	+
68	M.	—	—	—	120	M.	—	+	+
69	M.	—	—	—	121	F.	—	+	+
70	M.	—	—	—	122	F.	—	—	—

Table 40.—The Naked-eye Appearances of the Contents of the Alimentary Canal of 183 Tsetse Flies, with their Sex, and the Presence or Absence of a Larva—*continued*.

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
123	F.	—	+	+	154	M.	—	—	+
124	M.	—	+	+	155	M.	—	—	—
125	M.	—	—	—	156	M.	—	—	—
126	M.	—	—	+	157	M.	—	+	+
127	F.	—	—	—	158	M.	—	—	—
128	M.	—	—	+	159	F.	—	—	+
129	F.	—	—	+	160	M.	—	—	—
130	F.	—	—	+	161	F.	+	—	+
131	M.	—	+	+	162	M.	—	—	—
132	M.	—	+	+	163	M.	—	—	+
133	M.	—	+	+	164	M.	—	—	—
134	F.	—	+	+	165	M.	—	—	—
135	M.	—	—	—	166	M.	—	—	—
136	M.	—	—	—	167	F.	+	—	+
137	F.	+	—	+	168	F.	+	—	+
138	F.	—	—	+	169	F.	—	+	—
139	M.	—	—	—	170	F.	+	—	+
140	M.	—	—	+	171	M.	—	—	+
141	M.	—	—	+	172	M.	—	—	+
142	M.	—	—	+	173	F.	+	—	+
143	M.	—	+	+	174	M.	—	—	—
144	M.	—	—	—	175	M.	—	—	+
145	M.	—	—	+	176	M.	—	+	+
146	M.	—	—	—	177	F.	+	+	+
147	M.	—	+	+	178	F.	+	—	—
148	F.	+	—	+	179	F.	+	—	—
149	F.	+	—	+	180	F.	+	—	+
150	F.	—	—	+	181	F.	—	—	+
151	F.	—	—	+	182	M.	—	—	—
152	F.	—	—	+	183	M.	—	—	—
153	F.	+	—	+					

In the present experiment, the observer landed by canoe and made the paddlers sit round him; the fly-boys moved about amongst them and caught the flies as they came to feed. They were handed at once to the observer, who snipped off their heads, noted their sex, and roughly dissected out the alimentary canal, and, when it contained food-stuff, smeared it at full length on a slide. Notes were made as to the naked-eye contents of the canal and the question of pregnancy. The smears were fixed in the usual way and brought to the laboratory, where they were stained and examined minutely.

The total number of flies examined was 183, of which 104 (57 per cent.) were male and 79 (43 per cent.) were females; of the total number, 108 (59 per cent.) contained blood in a more or less digested state, and 75 (41 per cent.) contained no food-stuff. Out of the 79 females, 32 (40 per cent.) contained nearly fully-developed larvæ.

Table 41.—Shows the Nature of the Blood in the Interior of the Flies, and also the Number of Flies which contained Parasites.

No.	Mammalian. blood.	Non-mammal- ian blood.	Trypanosomes.	Halteridia.	No.	Mammalian blood.	Non-mammal- ian blood.	Trypanosomes.	Halteridia.
1	—	+	—	—	27	—	+	—	—
2	—	+	—	+	28	—	+	—	—
3	—	+	+	—	29	—	+	—	—
4	—	+	—	—	30	—	+	+	—
5	—	+	—	—	31	—	+	—	—
6	—	+	—	—	32	+	—	—	—
7	—	+	—	—	33	—	+	+	—
8	—	+	—	—	34	—	+	—	—
9	—	+	—	—	35	—	+	—	—
10	—	—	+	—	36	—	—	+	—
11	+	—	—	—	37	—	+	—	—
12	—	+	—	—	38	—	—	+	—
13	—	—	+	—	39	—	+	—	—
14	—	+	—	+	40	+	—	—	—
15	—	—	+	—	41	—	—	+	—
16	—	+	—	—	42	—	+	—	—
17	—	+	—	+	43	—	+	—	+
18	—	—	+	—	44	—	+	—	—
19	—	+	—	—	45	—	+	—	—
20	—	—	+	—	46	+	—	—	—
21	+	+	—	—	47	—	+	—	—
22	—	+	—	—	48	—	—	+	—
23	—	+	—	—	49	—	—	+	—
24	—	+	—	—	50	—	+	—	—
25	—	+	—	+	51	+	—	—	—
26	—	—	+	—	52	+	—	—	—

The above table shows that out of the original 183 flies examined, 52 (28 per cent.) contained blood which was recent enough to show the red corpuscles. Out of these 52, seven (13 per cent.) were from mammalian blood, whilst non-mammalian blood was present in 35 flies (67 per cent.). Trypanosomes were present in 14 flies (27 per cent.) and Halteridia in five (nearly 10 per cent.).

An attempt was made to separate the nucleated red blood corpuscles into avian and reptilian. The distinction was made by size only, it being found from the measurement of corpuscles from birds and reptiles that the average normal length was:—

Standard amphibian (crocodile), 15·4 microns.

Standard avian (horn-bill), 13·1 microns.

In examining the smears from the flies, the average was taken of 10 to 20 red cells, which seemed as natural as possible, and the average obtained in this way compared with the standard measurements. No allowance could be made for alterations due to digestive changes: these were presumed to be similar in all cases.

Table 42.—Shows the Average Measurements of the Nucleated Red Corpuscles and their Probable Origin.

No.	Average length of red cells, in microns.	Probably reptilian or amphibian.	Probably avian.
1	15.3	+	—
2	14.2	+	—
3	14.1	+	—
4	13.3	—	+
5	14.4	+	—
6	14.0	+	—
7	14.0	+	—
8	14.4	+	—
9	14.5	+	—
10	13.0	—	+
11	14.8	+	—
12	14.4	+	—
13	10.6	—	+
14	13.4	—	+
15	14.6	+	—
16	13.2	—	+
17	14.0	+	—
18	15.2	+	—
19	13.2	—	+
20	13.3	—	+
Totals ...		13	7

Remarks.—Only 20 of the flies contained nucleated blood which was recent enough to justify any deduction as to its origin. Out of these 20 flies, 13 had probably fed on a reptile or amphibian, and seven on a bird.

Table 43.—Showing the Result of the Examination of *Glossina palpalis* from “Crocodile Point.”

No. of flies.	Male.	Female.	F. containing larvae.	Intestine empty.	Intestine containing blood.	Mammalian blood.	Non-mammalian blood.	Non-mammalian blood.		Flagellates.	Halteridia.
								Avian.	Reptilian.		
183	104	79	32	75	108	7	35	7	13	14	5
Percentages ..	57	43	17.4	41	59	3.8	19.1	3.8	7	7.6	2.7

Conclusions.

Two hundred and twenty *Glossina palpalis* were caught on various parts of the Lake-shore, and at intervals extending over several months; they were examined about 24 hours after capture. The examination of their intestinal contents revealed the fact that about 27 per cent. contained the remains of blood, the majority of which was of mammalian origin.

In the second experiment, 183 *Glossina palpalis* were caught at one spot where the food supply was abundant—birds and crocodiles—and the flies were examined at once. A much higher percentage (nearly 60 per cent.) contained the remains of a blood meal. The blood in the majority of the flies had been obtained from birds or reptiles, and of these the reptilian blood was twice as frequent as the blood of birds. It must, however, be confessed that the method employed of distinguishing between avian and reptilian blood was crude and open to a wide margin of error.

17.—EXPERIMENTS TO ASCERTAIN IF *GLOSSINA PALPALIS* WILL FEED ON LIZARDS AND FROGS.

Several experiments of this sort were done, by letting out numbers of flies into a cage containing lizards and frogs, and by applying flies in cages to the under surface of the larger ones.

A number of chameleons were kept in the laboratory for the purpose of catching flies, &c. Occasionally an escaped tsetse fly would be seen greedily sucking the chameleon's blood, whilst the latter was stalking another tsetse fly on the window pane.

None of the experiments carried out were very successful, and generally a large proportion of the flies were devoured before they could attempt to feed. Even when the caged flies could bite in safety they did not do so; and this contrasted strongly with the avidity with which they feed on birds and on mammals. The instances of the escaped flies feeding on chameleons is, however, sufficient proof that flies at liberty will feed on small reptiles. The flies were also observed at least on one occasion sucking the blood of a lizard.

18.—EXPERIMENTS TO ASCERTAIN WHETHER FLAGELLATES FOUND NATURALLY IN LAKE-SHORE FLIES (*GLOSSINA PALPALIS*) ARE PATHOGENIC TO MAMMALS, BIRDS, AND REPTILES.

Minchin, Gray, and Tulloch* referred to the flagellates of wild flies as being of two distinct types—*Trypanosoma grayi* (Novy) and *Trypanosoma tullochi* (Minchin).

Trypanosoma grayi was described as possessing a large nucleus, which was always situated not far from the posterior end of the body. The micronucleus was described as being large, transversely elongated, and situated either close to or more usually anterior to the macronucleus. The trypanosome was filamentous in shape, and resembled the *Herpetomonas* group.

* 'Proceedings' of the Royal Society, B, vol. 78, 1906.

Trypanosoma tullocki, on the other hand, possessed a more rounded nucleus, placed near the middle of the body, and a small, usually circular blepharoplast, placed well behind the nucleus. It was less filamentous than *Trypanosoma grayi*, and more closely resembled the pathogenic trypanosomes.

The present Commission believed at first that they could distinguish between these two flagellates, and in the earlier experiments they were referred to and experimented with as different organisms. Latterly, however, as a result of more careful observation, and especially by drawing and measurement, attempts to differentiate them were given up, and the term *Trypanosoma tullocki* was dropped and all the wild flagellates were called *Trypanosoma grayi*.

The *Trypanosoma tullocki* referred to by the above-named authors, in our opinion, represents a stage in the development of certain parasitic trypanosomes, probably *Trypanosoma gambiense*.

Experiments to ascertain whether the flagellates found naturally in Lake-shore *Glossina palpalis* are pathogenic to mammals, birds and reptiles, were carried out by the Commission as follows:—

1. With flagellates of *Trypanosoma grayi* type.
2. With flagellates of a pathogenic type.

Experiments with Flagellates of Trypanosoma grayi type.

Experiment 47.

The intestines of a Lake-shore *Glossina palpalis*, which was heavily infected with *Trypanosoma grayi*, were injected into a monkey.

The blood of the monkey was examined for 56 days, with a negative result. The monkey was then killed and cultures were made on to Novy's medium:—

- (a.) Four tubes inoculated with defibrinated heart blood.
- (b.) Two with bone marrow.

Result.—Negative.

This experiment was repeated nine times, with monkeys (thrice), goats (twice), fowls (twice), and lizards (twice), with negative results.

Experiments with Flagellates of a Pathogenic type.

Experiment 1140.

The fore-gut, mid-gut, and hind-gut of a Lake-shore *Glossina palpalis*, in which swarms of flagellates were seen, were teased in normal saline and injected under the skin of a monkey.

The monkey developed *Trypanosoma gambiense* 11 days after.

Result.—Positive.

This experiment was repeated four times, with monkeys (thrice) and a goat (once), with negative results.

The following table is a summary of the experiments performed:—

Table 44.

Experiment No.	Type of flagellate.	Animal, etc., injected.	Result of injection.	Cultivation on Novy's medium.	
47	...	<i>T. grayi</i>	Monkey	—	Blood and bone marrow ; negative.
242	...	"	"	—	Blood and bone marrow ; negative.
1789	...	"	"	—	
1835	...	"	"	—	
1767	...	"	Goat	—	
1834	...	"	"	—	
212	...	"	Fowl	—	Blood ; negative.
254	...	"	"	—	Blood ; negative.
213	...	"	Lizard	—	
407	...	"	"	—	
1140	...	Pathogenic type.	Monkey	+	
1206	...	"	"	—	
1811	...	"	"	—	
1849	...	"	"	—	
1812	...	"	Goat	—	

Remarks and Conclusions.

Ten experiments were carried out with *Glossina palpalis*, caught on the Lake-shore, which contained trypanosomes corresponding to the type of *Trypanosoma grayi*. The intestines of 23 flies so infected were inoculated, subcutaneously, into monkeys, goats, fowls, and lizards. The results were all negative. There is, therefore, no evidence that any of these creatures are vertebrate hosts of *Trypanosoma grayi*. Lately, Dr. Kleine has brought forward evidence to show that *Trypanosoma grayi* is derived from the blood of the crocodile.

Five experiments were performed with *Glossina palpalis*, caught on the Lake-shore, infected with flagellates which resembled the developmental forms of pathogenic trypanosomes. One of these experiments was positive. In this positive experiment the intestines of a fly caught on the Lake-shore were found to be naturally infected with trypanosomes of a pathogenic type. These were inoculated into a monkey, and the animal developed *Trypanosoma gambiense* in its blood in 11 days. The four remaining experiments of this series were negative, possibly because the trypanosomes infecting the gut of the flies were in a non-virulent stage of their development.*

* See "Experiments to Ascertain if *Trypanosoma gambiense* During its Development within *Glossina palpalis* is Infective," and "Further Researches on the Development of *Trypanosoma gambiense* in *Glossina palpalis*," 'Proceedings' of the Royal Society, B, vol. 83, 1911, pp. 345 and 513 ; also pp. 54 and 34 of this Report.

19.—EXPERIMENTS TO ASCERTAIN IF THERE IS AN ULTRA-MICROSCOPICAL PHASE IN THE DEVELOPMENT OF *TRYPANOSOMA GAMBIENSE* IN *GLOSSINA PALPALIS*.

In order to ascertain if there is any ultra-microscopic form of *Trypanosoma gambiense* associated with its development within the fly, a series of experiments was performed in which batches of Lake-shore flies, 50 in each batch, were fed upon a *Trypanosoma gambiense*-infected monkey and, at intervals of 10, 13, 16, 17, 20, 25, and 34 days after their infected feed, the batches of flies were killed and their intestinal contents extracted and brayed in a mortar with normal saline solution. The emulsion thus prepared was filtered through a Berkefeld filter which had been proved impervious to *Micrococcus melitensis* suspended in broth. The filtrate from the infected intestines was then injected, subcutaneously, into monkeys.

The seven monkeys so inoculated remained in perfect health.

It seems, therefore, unlikely that there is any ultra-microscopical phase of trypanosomes during the period of development within the fly.

20.—EXPERIMENTS TO ASCERTAIN IF THERE IS ANY HEREDITARY TRANSMISSION OF *TRYPANOSOMA GAMBIENSE* BY *GLOSSINA PALPALIS*.

Experiment 1542.

Laboratory-bred flies, some of them presumably the offspring of infected flies, were fed on a healthy monkey before they were used for other experiments.

Date.	No. of flies applied.	Remarks.
1909.		
Aug. 22 ...	60	The blood of the monkey on which these flies were fed was examined for one month after last feed of flies, with a negative result.
" 23 ...	110	
" 24 ...	110	
" 25 ...	150	
" 26 ...	150	
" 27 ...	200	
" 28 ...	60	
" 29 ...	—	
" 30 ...	60	
" 31 ...	110	
Sept. 1 ...	110	
" 7 ...	50	
" 10 ...	50	
" 11 ...	90	
" 12 ...	40	
" 14 ...	45	
" 15 ...	123	
" 17 ...	50	
" 20 ...	110	
" 21 ...	60	
" 30 ...	110	
Total ...	1,858	

Result.—Negative.

Experiment 1683.

September 12, 1909, 50 laboratory-bred flies fed on a healthy monkey for 30 successive days.

October 25, 50 laboratory-bred flies fed on the monkey for 28 successive days.

November 6, 200 laboratory-bred flies fed on the monkey for one feed.

This monkey never showed trypanosomes in its blood.

(All these 300 flies were subsequently dissected and examined for flagellates, but none were found.)

Result.—Negative.

Experiment 1861.

One hundred laboratory-bred flies fed on a healthy monkey each day for 28 days.

The blood of the monkey was examined for one month after the last feed of the flies, but no trypanosomes were seen.

Result.—Negative.

The pupæ from which the laboratory-bred flies were hatched were obtained from parts of the Lake-shore where the flies were known to be infective.

Conclusion.

That there is no hereditary transmission of *Trypanosoma gambiense* by *Glossina palpalis*. It must be confessed, however, that these experiments are not sufficient, absolutely, to disprove hereditary transmission.

21.—EXPERIMENTS TO ASCERTAIN IF LABORATORY-BRED GLOSSINA PALPALIS WILL BECOME INFECTED WITH FLAGELLATES IF THEY ARE FED UPON HEALTHY OXEN, MONKEYS, OR FOWLS.

Experiment 1875.—100 laboratory-bred flies were fed daily for 30 days upon a healthy ox. At the end of this period the flies were dissected and the proboscis and gut examined, to ascertain if they had become infected with flagellates. All were negative.

Experiment 1669.—50 laboratory-bred flies were fed upon a normal monkey for 30 days and then dissected. Careful examination of the proboscis and gut failed to reveal the presence of flagellates.

Experiment 2018.—100 laboratory-bred flies were fed on a fowl for six days; they were subsequently fed daily upon a monkey for 42 days. Eighty-seven flies were dissected between the 11th and 55th days after the first feed on the fowl. All the flies proved negative for flagellates.

Experiment 2019.—100 laboratory-bred flies were fed on a fowl for six days, and subsequently on a monkey for 42 days. Eighty flies were dissected between the 11th and 55th days after the first feed on a fowl. All the flies proved negative for flagellates.

Experiment 2276.—200 laboratory-bred flies fed on a fowl for five days, and subsequently on a monkey for 33 days. One hundred and sixteen flies were dissected between the 23rd and 42nd days after the flies first feed on the fowl. All the flies proved negative for flagellates.

Conclusion.

Laboratory-bred flies are not infected with flagellates by feeding them upon healthy oxen, monkeys or fowls. This also affords more proof that there is no hereditary transmission of flagellates in *Glossina palpalis*.

22.—EXPERIMENTS TO ASCERTAIN IF LABORATORY-BRED *GLOSSINA PALPALIS* BECOME INFECTED WITH FLAGELLATES WHEN KEPT IN THE SAME CAGE WITH INFECTED WILD FLIES.

The tsetse flies caught on the shores of Lake Victoria were found to be naturally infected with flagellates (*Trypanosoma grayi*), in a proportion varying from 6 to 10 per cent.

Nothing was known as to how *Glossina palpalis* became infected with *Trypanosoma grayi* until Kleine* succeeded in infecting laboratory-bred flies with *Trypanosoma grayi* by feeding them upon a crocodile infected with a large trypanosome. He did not, however, believe it proved that *Glossina palpalis* is the host of the crocodile trypanosome, and considered that more experiments were needed.

Minchin,† in 1905, discovered and described encysted forms of *Trypanosoma grayi*, which he thought were destined to be voided with the dejecta of the fly and to be subsequently swallowed by another host, probably a vertebrate.

The following experiments were designed to ascertain if laboratory-bred tsetse flies become infected with flagellates when kept in the same cage with *Trypanosoma grayi*-infected flies caught on the Lake-shore.

Experiment 1806.—40 laboratory-bred flies were marked by amputation of a wing or a leg and put into the same cage with an equal number of Lake-shore flies. The cage of mixed flies was fed for one month on a healthy monkey. All the flies were dissected, including those that died during the experiment.

None of the laboratory-bred flies became infected with flagellates, whereas eight of the Lake-shore flies were found infected with *Trypanosoma grayi*.

Experiment 1857.—200 laboratory-bred flies were kept in intimate contact with Lake-shore flies, by placing the mosquito-netting sides of the cage containing the laboratory-bred flies in apposition to the sides of four cages containing many hundreds of

* *Deutsche Medizinische Wochenschrift*, Nov., 1909, pp. 1956-1958.

† Reports of the Sleeping Sickness Commission of the Royal Society, No. VIII., pp. 137-142.

wild flies. This contact was maintained for six weeks, so that the cage containing the laboratory-bred flies became thoroughly fouled with excrement from the *Trypanosoma grayi*-infected wild flies.

Seventy-six in all were dissected and their probosces and intestines were carefully examined. No flagellates were found. Forty-three of the Lake-shore flies were dissected and two of them were found to be infected with flagellates.

To illustrate further attempts to obtain experimental proof that tsetse flies can be infected with flagellates otherwise than by imbibing blood containing trypanosomes, an experiment with wild flies may be quoted. During the months of June and July, 1909, it was found that out of 130 wild flies from a certain locality on the Lake-shore, 6·9 per cent. of the flies were naturally infected with flagellates (*Trypanosoma grayi*). The infected intestines of these flies were smeared upon the skin of a monkey, then four cages of wild flies, each containing about 100 flies, were allowed to feed through the fouled skin of this animal. The flies were subsequently dissected at intervals of 6, 23, 28, 31 and 35 days after exposure to contamination, and 5·4 per cent. of these flies were found to harbour flagellates—*Trypanosoma grayi*—in the intestines and none in the proboscis.

To compare the result of the examination of flies before and after artificial contamination: the percentage of flies containing flagellates before artificial contamination was 6·9; after artificial contamination 5·4.

Conclusions.

1. Laboratory-bred flies do not become infected with flagellates after living for six weeks in captivity with Lake-shore flies which were known to be infected.

2. That the number of wild flies naturally infected is not increased by exposing these flies to artificial contamination with the gut-contents of infected flies.

3. This affords still further proof that there is no hereditary transmission of flagellates in *Glossina palpalis*.

23.—EXPERIMENTS TO ASCERTAIN WHETHER TRYPANOSOMA GAMBIENSE CAN BE TRANSMITTED TO SUSCEPTIBLE ANIMALS BY THE APPLICATION OF INFECTIVE BLOOD TO THE UNBROKEN MUCOUS MEMBRANE.

Experiment 525.

The inside of the nostrils of this monkey was painted with a soft camel's hair brush dipped in blood containing *Trypanosoma gambiense*.

The monkey was examined at frequent intervals for forty days.
Result.—Negative.

Experiment 526.

Monkey treated precisely as above.

Result.—Negative.

24.—EXPERIMENT TO ASCERTAIN WHETHER THE BLOOD OF MONKEYS
AFTER EXPERIMENTAL INOCULATION IS INFECTIVE BEFORE
TRYPANOSOMES ARE VISIBLE BY MICROSCOPIC EXAMINATION.

Experiment 1313.

July 12, 1909. Monkey was injected with a chopped-up fly which two hours previously had fed on a *Trypanosoma gambiense*-infected monkey.

July 14. *Forty-six hours after injection.* 1 c.c. of blood taken from the vein of monkey, 1313, and injected into monkey, 1319.

Monkey, 1313, showed no trypanosomes.

Monkey, 1319, did not become infected.

July 15. *Seventy-two hours after injection.* 1 c.c. of blood taken from monkey, 1313, and injected into monkey, 1338.

Monkey, 1313, showed no trypanosomes in its blood.

Monkey, 1338, *became infected* in seven days.

July 16. *Ninety-six hours after injection.* No trypanosomes are seen by microscopic examination in the blood of monkey, 1313.

July 17. *One hundred and twenty hours after injection.* 1 c.c. of blood removed from monkey, 1313, and injected into monkey, 1349, five days after the introduction of the chopped-up fly.

Monkey, 1313, showed trypanosomes in its blood this day (five days) by the microscope.

Monkey, 1349, became infected on July 22.

SUMMARY.

Date.	Infectivity by		Hours.
	Inoculation.	Microscopical examination.	
1909.			
July 12 ...	—	—	
" 13 ...	...	—	24
" 14 ...	—	—	46
" 15 ...	+	—	72
" 16 ...	...	—	96
" 17 ...	+	+	120

Conclusion.

The blood of monkey, 1313, was capable of giving rise to *Trypanosoma gambiense* infection in another monkey three days after inoculation and forty-eight hours before trypanosomes were seen in its blood on microscopical examination.

- 25.—EXPERIMENT TO ASCERTAIN IF A VISCID PREPARATION NAMED "TANGLEFOOT" WOULD, WHEN SMEARED ON A BLACK CLOTH, PROTECT THE WEARER FROM THE BITES OF *GLOSSINA PALPALIS*.

Experiment 2214.

February 20, 1911. "Tanglefoot" was smeared on a sheet of black cloth, which was worn by a member of the Commission, during a period of some hours on the Lake-shore, in a spot where *Glossina palpalis* were exceedingly numerous. A similar procedure was adopted in the case of a native at the same time and place.

The *Glossina palpalis* were undoubtedly attracted by the black cloth and smell of the "tanglefoot," but carefully avoided being caught. They would hover in front of the sticky surface for a second, test the ground by putting their feet lightly down and, finding the foothold unfavourable, would immediately fly off to alight on some part of the body of the observer, usually the face or neck, which they promptly bit.

House-flies and other small insects were readily caught by the "tanglefoot."

Conclusion.

"Tanglefoot" does not protect one from bites of *Glossina palpalis* on the shores of Victoria Nyanza.

D. TRYPANOSOME DISEASES OF DOMESTIC ANIMALS IN UGANDA.

26.—*TRYPANOSOMA PECORUM*, SP. NOV.*

Synonyms.

Trypanosoma dimorphon (Dutton and Todd).

Trypanosoma congolense (Brodén).

Trypanosoma confusum (Montgomery and Kinghorn).

Dr. Edington's trypanosome from Zanzibar (Bruce, Hamerton, and Bateman).

Trypanosome from Chai-Chai, Zambesi, Zululand (Theiler).

Trypanosome from Southern Rhodesia (Bevan).

Introduction.

As might be expected from the tropical nature of the country, Uganda suffers much from protozoal diseases, and as the wealth of the natives consists principally in cattle, goats, and sheep—the King and chiefs having huge herds—there is much loss.

To give an idea of the enormous mortality which must take place among the herds of cattle in Uganda, Sir Apolo Kagwa, K.C.M.G., the Prime Minister, may be quoted. He informed the Commission that during 1908 he had 1,396 cows, which had 2,021 calves, and of these calves 709 died—35 per cent. Further, of cows, bulls, and full-grown bullocks, there had died 351. This makes a total of 1,060 deaths in one year. The number of the whole herd is not given, but may be put down at 4,000. This means a yearly death-roll of about 25 per cent. During the same year the Government Transport Department in Entebbe lost 156 oxen between June and November.

During 1909 the Commission had the opportunity of investigating several outbreaks of trypanosome disease among domestic animals; one among the transport cattle at Entebbe (lat. 0° 3' N., long. 32° 30' E.); another at Kampala (lat. 0° 18' N., long. 32° 35' E.); a third at the Uganda Company's estate at Namukekera (lat. 0° 40' N., long. 32° 17' E.); a fourth at Mr. Walsh's farm at Kabula Muliro, a few miles west of Namukekera; and a fifth at the Mabira Rubber Estate (lat. 1° 50' N., long. 32° 40' E.).

The commonest trypanosome disease among cattle in Uganda is caused by a trypanosome of the *dimorphon* type, which it is proposed to call *Trypanosoma pecorum*. This species is probably the same as that which has been known by the name *Trypanosoma dimorphon* (Dutton and Todd), and is either identical with, or very similar to, Brodén's *Trypanosoma congolense*. The name *dimorphon* is a misleading one, and can only be accounted for by Dutton and Todd describing under one name two species of trypanosomes occurring in the same animal. No one, so far as we are aware, has re-discovered Dutton and Todd's *Trypanosoma dimorphon*, although many attempts to reconcile later observations with theirs have been made. Certainly the strain kept at the Liverpool School of Tropical Medicine under that name

* Reprinted, with an Addendum, from the 'Proceedings' of the Royal Society, B, vol. 82, 1910.

does not agree with the original description. The term *Trypanosoma dimorphon* must therefore disappear, since it was born of a misapprehension. But it will at once be said, if the name *Trypanosoma dimorphon* must go, why not call the species *Trypanosoma congolense*? The only reasons that can be given are, that *Trypanosoma congolense* is a local name and, therefore, not very suitable, and secondly, that if it comes to strict priority, then *Trypanosoma dimorphon* holds the field in spite of its misleading character and the error its authors fell into, because it cannot be denied that there is a strong feeling that *Trypanosoma dimorphon* and *Trypanosoma congolense* are one. At the same time it must also be granted that there are others who are strongly of opinion that *Trypanosoma dimorphon* and *Trypanosoma congolense* are distinct species. Under these circumstances it seems best to unite the old names under a new one, and *Trypanosoma pecorum* seems appropriate enough, as this trypanosome disease is peculiarly one of herds. Montgomery and Kinghorn have lately proposed the name *confusum*, in order to get out of the difficulty; but this name has been used for a trypanosome already, and it is, moreover, an awkward term.

It must then be understood that in the species *Trypanosoma pecorum* we include *Trypanosoma dimorphon*, *Trypanosoma congolense*, the trypanosome discovered in Zanzibar by Edington and described in the 'Proceedings' of the Royal Society,* that from Portuguese East Africa and Zululand described by Theiler, and the species found in Northern Rhodesia by Montgomery and Kinghorn, and in Southern Rhodesia by Bevan.

The other species of trypanosomes found in the blood of cattle in Uganda were *Trypanosoma gambiense*, *Trypanosoma brucei*, *Trypanosoma vivax*, *Trypanosoma cazalboui*, and *Trypanosoma nanum*. These names, however, may require to be reconsidered.

Distribution in Uganda.

Cattle suffering from *Trypanosoma pecorum* were sent to the Sleeping Sickness Commission's laboratory at Mpumu from Entebbe, Kampala, Namukekera, Kabula Muli, and Mabira. It is probably widely distributed throughout Uganda. It was also found in a horse which had arrived in Nairobi, British East Africa, from Abyssinia. The Commission is indebted to Mr. Sturdy, Principal Veterinary Officer, for the opportunity of studying this trypanosome. It is not known where the horse became infected, but it must have been at some point between Nairobi and the Abyssinian border.

Morphology of Trypanosoma pecorum.

(a) Living, Unstained.

This trypanosome, when observed in a preparation of fresh blood, is seen to remain at or near the same spot in the field, that is to say, it is non-translatory. It is, however, active and restless, the body quivering rapidly, and the undulating membrane and flagellum keeping up a constant vibratory motion.

* B, vol. 81, p. 14; also Reports of the Sleeping Sickness Commission of the Royal Society, No. X., p. 12.

As a rule, it moves with the flagellar end in front. The contents of the cell are homogeneous, except for a vacuole at the posterior extremity.

A marked characteristic of this species is that it exhibits alternating periods of quiescence and activity. When quiescent it is usually invisible, as it has a habit of burying itself under small collections of red blood corpuscles.

(b) *Fixed and Stained.*

Method of Staining.—Giemsa's method, as described in a former paper,* was used.

Length.—The same method of measuring was used as described in the same paper.

Breadth.—Without the undulating membrane the average is about 2 microns, with the membrane about 3 microns.

Shape.—This trypanosome when stained is short and stout in form. The posterior extremity is blunt, or rounded, or pointed and angular. The anterior end is narrower. The undulating membrane is fairly well developed, more so, perhaps, than in *Trypanosoma nanum*. The flagellum arises near the micronucleus, and passes along the edge of the undulating membrane. There is no free flagellum.

Contents of Cell.—Generally homogeneous. Sometimes granules are seen which take on a chromatin stain, and are situated anterior to the nucleus.

Nucleus.—Is oval in shape, and situated about the middle of the body.

Table 45.—Measurements of *Trypanosoma pecorum*.

No. of experiment.	Animal.	Day of disease.	Method of fixing and staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
82	Ox	—	Osmic acid and Giemsa	11·7	14·0	10·0
505	"	42	"	12·3	15·0	10·0
—	"	—	"	13·2	15·0	11·0
593	Sheep	50	"	14·5	17·0	10·0
44	Monkey	31	"	12·2	14·0	10·0
559	"	14	"	12·7	16·0	11·0
461	Dog	44	"	15·3	18·0	13·0
543	"	10	"	14·3	16·0	11·0
1406	"	31	"	13·7	16·0	11·0
551	Rat	19	"	12·6	18·0	8·0
626	"	—	"	12·8	15·0	10·0
398	Mouse	28	"	14·6	16·0	13·0
398	"	36	"	13·1	17·0	10·0
				13·3	18·0	8·0

* 'Proceedings' of the Royal Society, B, vol. 81, p. 16; also Reports of the Sleeping Sickness Commission of the Royal Society, No. X., p. 15.

Micronucleus.—Small and round, and situated near to, but not at, the posterior extremity.

Undulating Membrane.—Is simple, but fairly well developed.

Flagellum.—There is no free flagellum. In very rare cases, where there is an appearance of a free flagellum, the trypanosome will be found to be dividing.

As it is difficult to gain an idea of the general appearance of a trypanosome, a series of coloured drawings by Lady Bruce, R.R.C., is also given to supplement the written description (Plate 2). The trypanosomes have been stained by Giemsa's method, and drawn at a magnification of 2,000. By referring to them, the shape, disposition of the micronucleus, and other parts of the structure of *Trypanosoma pecorum*, will be more readily understood.

As will be seen from Table 47, the disease set up in domestic animals by *Trypanosoma pecorum* is a serious and fatal one.

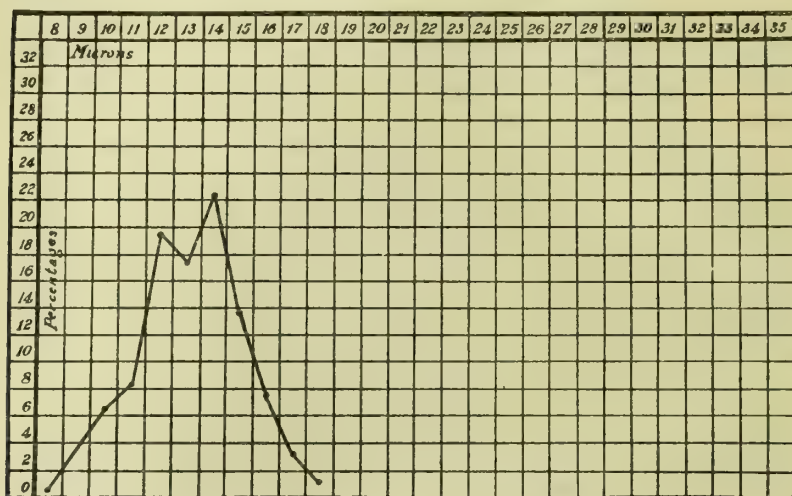
Cattle, goats, sheep, monkeys, dogs, rats, and mice are susceptible. Guinea-pigs, on the other hand, are refractory. Horses, mules, donkeys, and rabbits were not available at Mpumu, so that, unfortunately, it is not possible to say whether they are inoculable or not. In regard to guinea-pigs it would be interesting to know whether a series of inoculations into rats or rabbits would make the *Trypanosoma pecorum* also pathogenic for them.*

Table 46.—Represents the distribution in respect to length of 260 individuals of *Trypanosoma pecorum*.

Experiment.	Microns.											Average, in microns.
	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	
82	—	—	3	4	9	3	1	—	—	—	—	11·7
505	—	—	2	2	9	4	1	2	—	—	—	12·3
—	—	—	—	2	3	6	6	3	—	—	—	13·2
593	—	—	1	—	2	1	3	7	5	1	—	14·5
44	—	—	4	2	5	3	6	—	—	—	—	12·2
559	—	—	—	2	9	4	4	—	1	—	—	12·7
461	—	—	—	—	—	5	4	1	2	6	2	15·3
543	—	—	—	2	—	2	6	6	4	—	—	14·3
1406	—	—	—	1	5	2	4	6	2	—	—	13·7
551	1	—	3	3	3	3	3	2	1	—	1	12·6
626	—	—	3	2	3	2	7	3	—	—	—	12·8
398	—	—	—	—	—	1	9	6	4	—	—	14·6
398	—	—	1	1	3	9	4	—	1	1	—	13·1
Totals ...	1	—	17	21	51	45	58	36	20	8	3	—
Percentages }	0·4	—	6·5	8·1	19·6	17·4	22·3	13·9	7·6	3·1	1·1	—

* This was successfully done, vide Addendum to this paper, p. 139.

Chart giving curve of the distribution, by percentages, in respect to length, of *Trypanosoma pecorum*.



Animals susceptible to *Trypanosoma pecorum*.

Table 47.

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
CATTLE.				
82	Natural infection.	?	2	Died 2 days after arrival.
110	"	?	243	Treated with lithium antimonyl tartrate.
230	"	?	4	Died 4 days after arrival.
357	"	?	3	" 3 "
358	"	?	1	" 1 day after arrival.
359	"	?	60	Treated with arsacetin.
360	"	?	14	Died of <i>Trypanosoma pecorum</i> .
391	"	?	313	" "
475	"	?	9	Died " 9 days after arrival.
476	"	?	2	" 2 "
477	"	?	13	" 13 "
482	"	?	5	" 5 "
483	"	?	34	" 34 "
505	"	?	265	Killed.
550	"	?	—	Treated with arsenic; still alive after 254 days.
1459	"	?	43	Died 43 days after arrival.
1560	"	?	46	" 46 "
1731	"	?	79	Killed.
1733	"	?	76	Died 76 days after arrival.
1735	"	?	79	Killed.
1736	"	?	79	" "
1737	"	?	84	Died of <i>Trypanosoma pecorum</i> .
97	Ox	9	46	" "
447	"	8	287	Killed.

* Duration includes the days of incubation; it dates from the day of infection.

*Animals susceptible to Trypanosoma pecorum.*Table 47—*continued.*

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
CATTLE— <i>cont.</i>				
1463	Ox	7	35	Died of <i>Trypanosoma pecorum</i> .
1464	"	7	14	
1225	Monkey	—	—	Died " 8 days after inoculation ; bitten by snake. Never showed trypanosomes.
1357	Dog	6	15	Died of <i>Trypanosoma pecorum</i> .
1358	"	6	18	" "
1359	"	6	22	" "
1363	"	6	15	" "
1364	"	6	38	" "
1365	"	6	29	" "
	Average	6·7	63	
GOAT.				
1005	Ox	—	—	Never showed trypanosomes ; still alive after 72 days.
1006	"	14	44	Cause of death doubtful.
1404	"	9	41	" "
1405	"	—	—	Never " showed trypanosomes ; under observation 31 days.
633	Monkey	12	51	Cause of death doubtful.
	Average	11·6	45	
SHEEP.				
697	Ox	21	—	Still alive after 170 days.
632	Monkey	8	43	Cause of death doubtful.
593	Dog	19	168	Died of <i>Trypanosoma pecorum</i> .
	Average	16	105	
MONKEY.				
44	Ox	14	35	Died of <i>Trypanosoma pecorum</i> .
49	"	10	16	" "
376	"	11	56	" "
683	"	10	61	" "
1740	"	11	—	Still alive after 45 days.
719	Sheep	21	—	" 216 "
350	Monkey	10	181	Died of <i>Trypanosoma pecorum</i> .
459	"	6	80	" "
1000	"	16	45	" "
460	Dog	11	75	Killed for cultivation experiments.
559	"	12	39	Died of <i>Trypanosoma pecorum</i> .
560	"	14	32	" "
581	Rat	13	86	" "
	Average	12·3	64	

* Duration includes the days of incubation ; it dates from the day of infection.

Animals susceptible to Trypanosoma pecorum.

Table 47—continued.

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dog.				
543	Horse	9	26	Died of <i>Trypanosoma pecorum</i> .
148	Ox	10	46	" "
349	"	22	98	" "
552	"	—	—	Re-injected after 17 days.
1007	"	—	—	Found dead after 14 days.
1406	"	21	29	Killed.
1407	"	—	—	Died ; under observation 31 days.
1193	Monkey	8	16	Died of <i>Trypanosoma pecorum</i> .
433	Dog	7	44	" "
434	"	9	58	" "
461	"	6	40	" "
1544	"	10	21	" "
552	Rat	—	—	Still alive after 49 days.
Average		11·3	42	
GUINEA-PIG.				
685	Ox	—	—	Still alive after 96 days.
1162	"	—	—	" 36 "
1163	"	—	—	" 36 "
1164	"	—	—	" 36 "
1647	"	—	—	Died ; under observation 29 days.
628	Monkey	—	—	Still alive after 104 days.
1002	"	—	—	" 50 "
566	Dog	—	—	" 78 "
WHITE RAT.				
397	Ox	17	39	Died of <i>Trypanosoma pecorum</i> .
551	"	16	18	Killed for cultivation experiments.
684	"	16	32	Died of <i>Trypanosoma pecorum</i> .
699	"	—	—	Experiment stopped after 57 days.
1646	"	12	16	Died of <i>Trypanosoma pecorum</i> .
626	Monkey	12	22	Killed for cultivation experiments.
1001	"	13	23	Died of <i>Trypanosoma pecorum</i> .
455	Rat	9	12	" "
729	"	11	21	" "
1708	"	8	12	" "
Average		12·6	21	
MOUSE.				
686	Ox	—	—	Experiment stopped after 59 days.
398	Monkey	26	41	Died of <i>Trypanosoma pecorum</i> .
627	"	12	26	" "
454	Mouse	6	12	" "
Average		14·7	26	

* Duration includes the days of incubation ; it dates from the day of infection.

Theiler describes a trypanosome from Chai-Chai, near the mouth of the Limpopo, in Portuguese East Africa; from the mouth of the Zambesi; and also from Zululand, which resembles the one under discussion, in not infecting guinea-pigs; and considers that this one fact is sufficient for the creation of a new species. We cannot agree with him in this, as there is no practical importance, except in the laboratory, in the fact that the guinea-pig is insusceptible; and, moreover, until more experiments have been made, we cannot be sure that under certain conditions of dosage or passage through the smaller animals the guinea-pig will remain refractory.

The important facts in regard to this species are, that man is not susceptible, but that the valuable domestic animals are, and that in these animals the disease is, as a rule, a fatal one.

As long as our knowledge of trypanosomes is limited it seems better to group them under as few names as possible. As knowledge grows, and as fundamental differences emerge, then it will be time to define them more strictly. As far as our present knowledge goes, the morphology of *Trypanosoma dimorphon*, *Trypanosoma congolense*, the Uganda, Zanzibar, Chai-Chai, Zambesi, Zululand, and Rhodesian strains is identical; these trypanosomes affect the same important domestic animals; the carrier is probably or may be the same, though this is not known, and therefore these various forms should, for the present, be grouped under one name; and for certain reasons given above we propose the name *Trypanosoma pecorum* for this group.

Disease set up in Cattle by Trypanosoma pecorum.

It is unnecessary in this paper to describe in detail the symptoms which can be noted during life, or the pathological changes set up in the organs of cattle by this trypanosome. It will be sufficient to say that the main symptoms are emaciation, anæmia, and progressive weakness, and that the principal *post-mortem* appearances are those due to anæmia.

Incubation.—As is probably true of most trypanosome diseases in susceptible animals, the period of time which elapses between the infection of the animal and the appearance of the trypanosomes in sufficient numbers in the peripheral blood to be seen by the microscope, is a short one: in this case, an average of 6·7 days.

Duration.—Of the course and duration of this disease in cattle little, unfortunately, is known. Most of the cattle which came under observation at the laboratory of the Commission at Mpumu were already sick when they arrived, and it was, as a rule, impossible to know when they had been infected. By referring to Table 47 it will be seen that 22 naturally-infected cattle were under observation. Four of them lived, on an average, nine months. Of these four, one, treated with arsenic, was still alive and apparently healthy in December, 1909, one was killed, one had been treated with lithium antimonyl tartrate, and one died without treatment at the end of 313 days. It is therefore impossible to say from the insufficient data at our disposal whether spontaneous cure ever takes place in this disease in cattle.

When we turn to the cases of cattle which were inoculated on the hill, and were therefore under observation from the beginning, we are struck by the rapid course of the disease. One animal certainly lived 287 days, but the remainder died, on an average, in 26 days from the date of infection. Most of these oxen were inoculated with a strain of this trypanosome which had caused a rapidly fatal epidemic among a herd of milch cattle belonging to Mr. Walsh, at Kabula Muliro. In the short space of one month 24 of Mr. Walsh's cattle died, and in two months 34 had died out of a herd of about 300 head.

It may therefore be concluded that *Trypanosoma pecorum* sets up a rapid and fatal disease in cattle.

Disease set up in Goats and Sheep by Trypanosoma pecorum.

The number of cases of this disease in goats and sheep which came under observation is too small to draw any conclusions from. At Mpumu the goats and sheep were not satisfactory experimental animals, as many of them died from some unknown cause. It was thought that as these animals usually lived in the valleys, and were often housed in their owner's hut during the night, the exposure on the top of the hill had a bad influence. One sheep was still alive after 168 days, and it is probable that most of the goats and sheep would have lived much longer if they had been kept under more favourable conditions.

Disease set up in the Smaller Laboratory Animals.

It is not necessary to describe in detail the action of *Trypanosoma pecorum* on the monkey, dog, and smaller laboratory animals, as a reference to Table 47 will show the average periods of incubation and duration. It will be seen that this is a fairly rapid and fatal disease in the dog, white rat, and mouse. In the monkey the average duration is about two and a-half months.

The Carrier of Trypanosoma pecorum.

Glossinæ.—From experiments made in the laboratory at Mpumu it seems probable that *Glossina palpalis* is capable of acting as a carrier of this trypanosome. Four experiments were made with ordinary wild Lake-shore flies, and of these one was successful. Four were also made with laboratory-bred flies, and one again came off. The latter experiment, however, with laboratory-bred flies was not free from doubt; but from the other, which seemed free from doubt, it appears that *Trypanosoma pecorum* can develop in *Glossina palpalis* and infect a healthy animal after a period of 21 days. More observations are required. It may be noted that in no instance did *Trypanosoma pecorum* appear in the blood of animals upon which freshly-caught Lake-shore tsetse flies had been fed. These flies were found to be

naturally infected with *Trypanosoma gambiense* and *Trypanosoma vivax*. This is an argument, though a small one, that *Glossina palpalis* is not the common or chief carrier of *Trypanosoma pecorum*.

Tabanidæ.—There is some circumstantial evidence available to show that *Trypanosoma pecorum* is carried by the *Tabanidæ*. In the valleys round Mpumu Hill, so far as we are aware, there are no tsetse flies at any time of the year.* As a rule, there are a few *Tabanidæ*. The cattle belonging to the Commission went down to the foot of the hill every morning to graze, and returned to their kraal on the top at sunset. Half of the herd went to the east of the hill and half to the west. On both sides there was a small valley or glen, through which ran a small stream. In these valleys during the year, as a rule, a *Tabanus* or two or a *Hæmatopota* could be seen, but they were in small numbers. Now it is a curious fact that at certain times of the year enormous numbers of *Tabanidæ* will suddenly appear in places where only a few are, as a rule, to be found. For example, Mr. Brown, at Mabira, who was collecting the biting flies of his district, wrote on March 14, 1909, that the *Tabanidæ*, which for months had been scarce, were then swarming everywhere in countless numbers, and he afterwards wrote that this invasion lasted about a month. The particular species which appeared at Mabira at this time was *Tabanus socialis* (Walk.).

So, in the same way, at Mpumu, the *Tabanidæ*, which had been rare, suddenly appeared in swarms. They were first seen in the valley to the west of the hill in September, 1909, and a month later in the valley to the east. Soon after this the cattle which had shown no signs of disease during the previous year were found to be suffering from *Trypanosoma pecorum*. Those which grazed in the valley to the west were the first to be affected, and afterwards those which grazed to the east of the hill. The species of *Tabanidæ* in this case was *Tabanus secedens* (Walk.). In both groups of cattle there were cases of *Trypanosoma pecorum* disease, so that the *Tabanidæ* had a reservoir from which to draw the virus.

Another sudden epidemic of *Trypanosoma pecorum* disease occurred on Mr. Walsh's farm at Kabula Muliro, where, as stated above, 34 milch cattle died within two months in a herd of 300. The evidence is all against this epidemic having been caused by tsetse flies. During February and March, and again later in the year, during August and September, as many as 100 fly-boys were engaged scouring this district for biting flies. *Tabanidæ*, *Hæmatopota*, and *Chrysops* were brought in, but not a single tsetse, although a reward of 5 rupees was offered for each specimen. The commonest *Tabanus* in this district during August was *variatus* (Walk.).

It may, therefore, in our opinion, be concluded that the trypanosome disease caused by *Trypanosoma pecorum* can be carried

* After this was written a few *Glossina palpalis* were found on the banks of a small stream near the foot of the hill.

from sick to healthy animals without the aid of *Glossinæ*, but what other species of fly, if any, acts as carrier is merely a matter of speculation at present.

Stomoxys.—Montgomery and Kinghorn state that they have strong evidence against this genus. At Mpumu several experiments were made to attempt to settle this question, but although they were persevered in for months, they remained negative. *Stomoxys* are so numerous in every part of the country all the year round that it seems inconceivable that they can act as carriers. From October, 1908, until the following September, although numerous cases of cattle with *Trypanosoma pecorum* in their blood grazed all day long with healthy cattle, yet not a single case of infection took place. The *Stomoxys* were exceedingly numerous all this time, forming a small cloud of flies round the cattle, and passing constantly from one animal to another, being driven hither and thither by the rapidly-swishing tails. This is a natural experiment on a large scale.

It will therefore require very convincing proof to bring this Commission to the belief that *Stomoxys* are carriers of this disease.

The subject of the carrier of *Trypanosoma pecorum* must remain in this unsatisfactory state for the present, but it is hoped that experiments which are at present being carried out at Mpumu may throw some light on this important part of the subject.*

Cultivation of Trypanosoma pecorum.

One difficulty experienced at Mpumu in attempts to cultivate the various trypanosomes was that rabbits were not available to supply the blood for Novy and MacNeal's medium. The blood of rats, goats, and oxen was used; but in making the cultivation of trypanosomes a factor in their diagnosis uniformity must be of the first importance.

Another difficulty was the frequency of contamination of the tubes. This was, perhaps, to be expected in a laboratory on the top of a hill in the tropics, with very free ventilation.

Trypanosoma pecorum grows fairly readily on blood-agar medium. At the end of 24 hours clumps may be seen containing many trypanosomes, with their flagellar ends directed outwards and in active motion (Plate 3, Fig. 1). The individual flagellates have irregularly-shaped granules of chromatin scattered through their body substance, and also many brightly-refractile vacuoles. After 48 hours' culture every field contains many active trypanosomes, and also small clumps composed of 10, 20, or more members (Plate 3, Fig. 2).

* The results of these researches are recorded in the paper entitled "Experiments to Ascertain if certain *Tabanidæ* act as the Carriers of *Trypanosoma pecorum*" (see 'Proceedings' of the Royal Society, B, vol. 83, 1911, p. 349, and p. 175 of this Report).

After six days the trypanosomes are still very active; they vary much in size and shape, from the plasmodial to the elongated, flagellated, highly active trypanosome. After this they seem to degenerate, and in a few days living trypanosomes can no longer be found in the tube.

This description approaches to some extent that of the cultural characters of Dr. Edington's trypanosome from Zanzibar and *Trypanosoma dimorphon*, but there is not that extraordinary growth which was described as covering several fields of the microscope. Whether this was due to the difference in the composition of the blood medium, or to the higher temperature at Mpumu, it is impossible at present to say.

By comparing the coloured plates of this trypanosome and the one from Zanzibar, a certain resemblance will be seen, but how far this should be taken as a factor in the diagnosis is difficult to say.

Conclusions.

1. *Trypanosoma pecorum* is an important trypanosome disease of domestic animals in Uganda.

2. It is similar in morphology, action on animals, and cultural characters, to the *Trypanosoma dimorphon* described by Laveran and Mesnil, and to Dr. Edington's trypanosome from Zanzibar, described in the 'Proceedings' of the Royal Society.*

3. The carrier is unknown.

Addendum.

The enquiry as to whether a series of inoculations into rats or rabbits would make *Trypanosoma pecorum* pathogenic to guinea-pigs, was pursued after the publication of the above paper. The following experiments show the number of passages and the result:—

No. of experiment.	Animal.	Source of virus.	Result.
2142	Monkey ...	Ox, natural infection	+
2199	Rat ...	Monkey, 2142 ...	+
2270	Calf...	Rat, 2199 ...	+
2406	Rat ...	Calf, 2270 ...	+
2462	Guinea-pig ...	Rat, 2406 ...	+

Remarks.—Guinea-pig, Experiment 2462, showed *Trypanosoma pecorum* in its blood 22 days after inoculation from an infected rat, and died on the 29th day after inoculation. The

* B, vol. 81, p. 14.

strain of *Trypanosoma pecorum* was subsequently carried on by passage through guinea-pigs.

The following experiments give the results of these passages:—

Experiment 2548.

Guinea-pig inoculated, subcutaneously, on June 9, 1910, with blood of guinea-pig, Experiment 2462, showed *Trypanosoma pecorum* in its blood for the first time eight days after inoculation, and died on the 11th day of the disease.

Experiment 2567.

Guinea-pig inoculated from guinea-pig, Experiment 2548. It showed *Trypanosoma pecorum* in its blood for the first time seven days after inoculation, and died on the 10th day.

This strain was brought to England and still retains its virulence to guinea-pigs.

DESCRIPTION OF PLATES.

PLATE 2.

This plate represents the general appearance of *Trypanosoma pecorum* in stained preparations. Note the rounded, blunt, or angular shape of the posterior extremity; the small, round micronucleus and vacuole placed near this end; the oval nucleus; and the absence of a free flagellum, except in dividing forms. Stained Giemsa, $\times 2000$.

PLATE 3.

FIG. 1.—An aggregation, or clump, of *Trypanosoma pecorum* after 24 hours' growth in blood-agar. Stained Giemsa, $\times 2000$.

FIG. 2.—The same after 48 hours. Stained Giemsa, $\times 2000$.

FIGS. 3, 4, and 5.—*Trypanosoma pecorum* after 3 days' growth. Stained Giemsa, $\times 2000$.

FIGS. 6 and 7.—*Trypanosoma pecorum* after 4 days' growth. Stained Giemsa, $\times 2000$.

FIGS. 8 and 9.—*Trypanosoma pecorum* after 8 days' growth. Stained Giemsa, $\times 2000$.

FIGS. 10, 11, and 12.—*Trypanosoma pecorum* after 10 days' growth. Stained Giemsa, $\times 2000$.

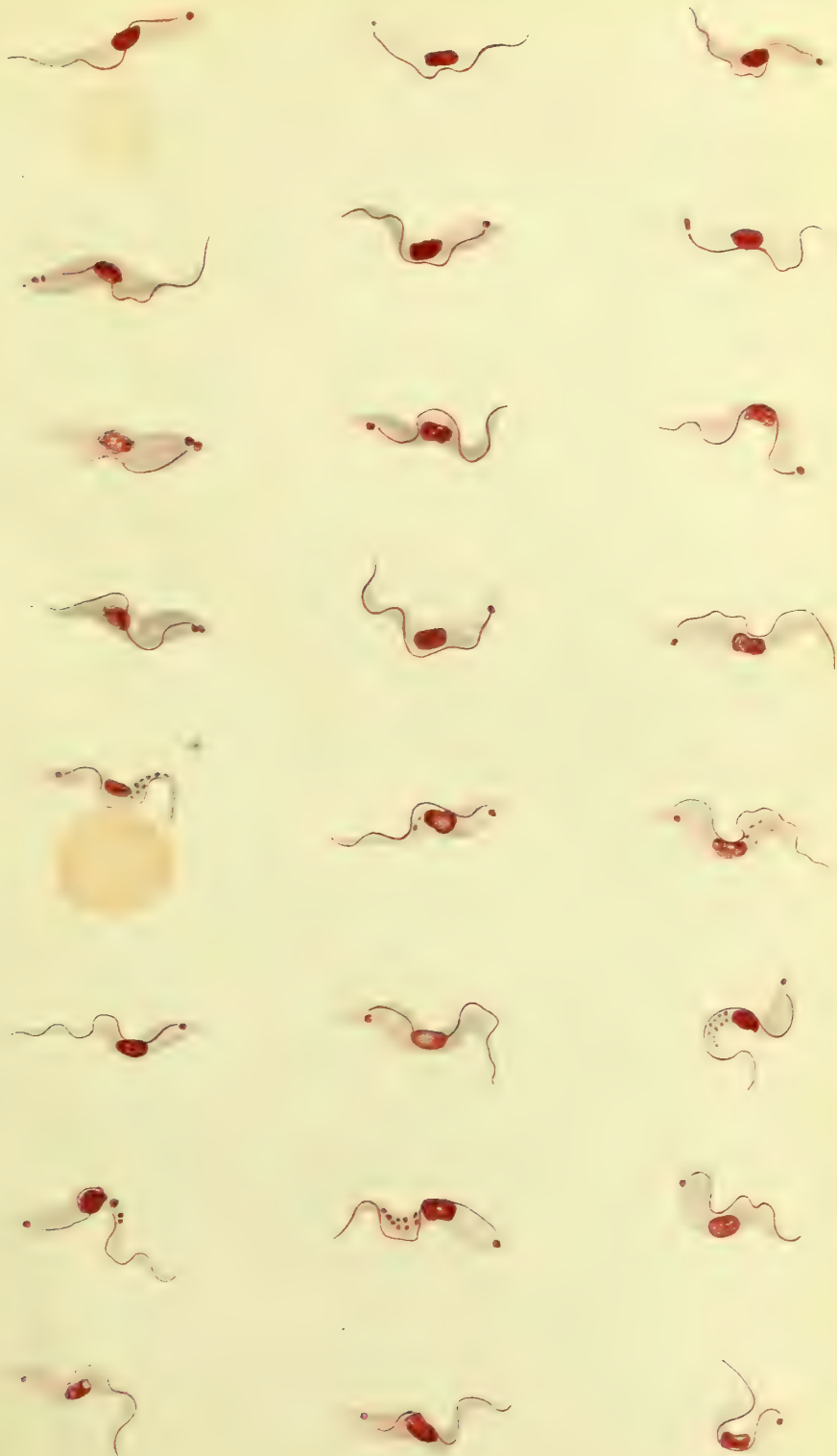
27.—TRYPANOSOMA BRUCEI (PLIMMER AND BRADFORD).*

Synonym: "Jinja trypanosome," Sleeping Sickness Commission, Royal Society, 1903.

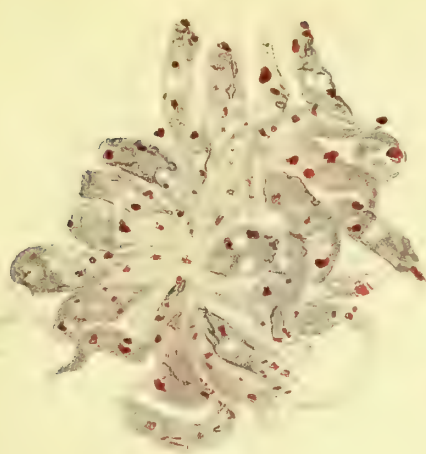
Introduction.

This species was only met with on one occasion during the work of the Commission in 1909. This was in the blood of an ox from

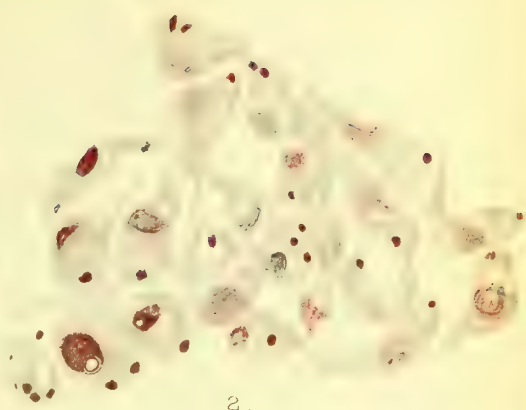
* Reprinted from the 'Proceedings' of the Royal Society, B, vol. 83, 1910.







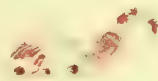
1.



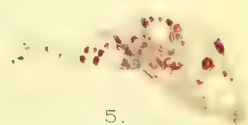
2.



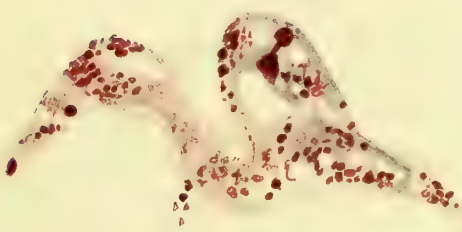
3.



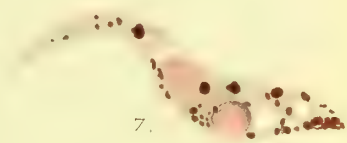
4.



5.



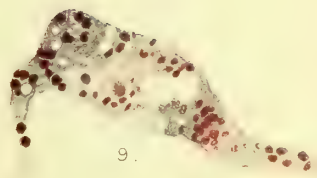
6.



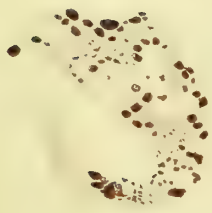
7.



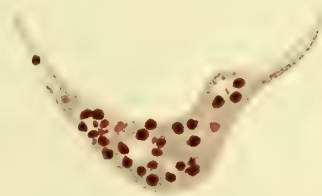
8.



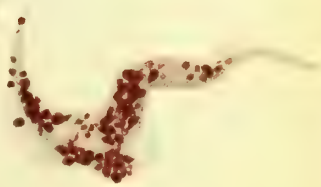
9.



10.



11.



12.



the Mabira Rubber Estate (latitude $0^{\circ} 30' N.$, longitude $32^{\circ} 55' E.$). The manager wrote that the animal came from the Bukedi District, about 100 miles to the north (latitude $1^{\circ} 50' N.$, longitude $32^{\circ} 40' E.$). Not much is known of this district, as it has only recently come under administration, and therefore it is impossible to say whether the ox was infected in Bukedi or on the journey south.

This is the species of trypanosome which was first discovered by Bruce, in 1894, in Zululand, to be the cause of Nagana, or tsetse-fly disease. During the work of the Sleeping Sickness Commission of the Royal Society in 1903, it was also met with in a herd of cattle from the same district of Bukedi, and then described as the "Jinja trypanosome."* It is impossible to name with any certainty the trypanosome seen in 1903, which affected the horses, camels, and dogs of the Abyssinian Boundary Commission. This was described as the "Abyssinian trypanosome." Its morphology, as given in the coloured plate,† shows it to be similar to *Trypanosoma brucei*, so that in all likelihood it was either this species or the closely related *Trypanosoma evansi*. As camels were infected, it was more probably the latter.

In regard to the third kind of trypanosome found in 1903, and referred to as the "Mule trypanosome," no opinion can at present be given.

As to the remaining trypanosome seen in 1903, and known as "Pordage's ox trypanosome,"‡ an examination of the old specimens at once disclosed its identity. It is *Trypanosoma vivax* (Ziemann); and this accounts for the fact that the blood of the ox injected into a monkey and a dog had no effect, these animals being refractory to *Trypanosoma vivax*.

Morphology of Trypanosoma brucei, Uganda, 1909.

This species is interesting on account of its well-marked dimorphism, in which *Trypanosoma gambiense* resembles it. There are two varieties—the long and slender with free flagellum, and the short and stumpy without free flagellum. The shortest are only 13, and the longest 35 microns in length.

In this paper the long and slender are considered to be those which measure from 25 to 35 microns; the short and stumpy from 13 to 21 microns. Those trypanosomes which measure 22, 23 or 24 microns are called intermediate. The general character of these forms may be given, shortly, as follows:—The long and slender have a narrow extended posterior extremity, an elongated oval nucleus, a well-developed undulating membrane thrown into many waves, folds or plications, and a long free flagellum (see

* Reports of the Sleeping Sickness Commission of the Royal Society, No. VI, p. 112.

† *Ibid.*, No. VI, plate 2, p. 113.

‡ *Ibid.*, No. IV, p. 48.

Plate 5, figs. 1 to 5). The short and stumpy, on the other hand, have a rounded posterior extremity, somewhat like the head of a turtle, a round or oval nucleus lying transversely to the long axis of the trypanosome, a well-developed undulating membrane thrown into bold folds, and no free flagellum (see Plate 5, figs. 11 to 16). The intermediate have a short free flagellum, a posterior extremity resembling that of the short and stumpy form but narrower, an oval nucleus, and a well-developed undulating membrane (see Plate 5, figs. 6 to 10).

(a) *Living, Unstained.*

No observations were made in Uganda on this species of trypanosome in the living, unstained condition.

(b) *Fixed and Stained.*

The blood films were fixed, stained, and measured as described in the 'Proceedings' of the Royal Society.*

Length.—The following table gives the length of this species as found in Uganda, in the ox, monkey, dog, guinea-pig, and rat. Only one trypanosome was found in the blood of the original Mabira ox. It measured 24 microns. In the monkey only 11 trypanosomes could be found. In all the others, 20 trypanosomes have been drawn and measured. These were taken as they came, except that dividing forms were passed by.

Table 48.—Measurements of *Trypanosoma brucei*, Uganda strain, 1909.

Date.	No. of experiment.	Animal.	Day of disease.	Method of fixing and staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1909.							
Aug. 12	1461	Ox	Not known	Osmic Acid and Giemsa.	24.0	24.0	24.0
Sept. 30	1627	Monkey	26th	"	24.8	30.0	19.0
Aug. 26	1479	Dog	41st	"	26.5	32.0	22.0
" 30	1479	"	45th	"	22.0	29.0	16.0
Sept. 30	1479	"	76th	"	23.5	34.0	20.0
Oct. 11	1644	Guinea-pig.	33rd	"	20.7	34.0	15.0
" 18	1644	"	40th	"	27.1	34.0	17.0
Sept. 9	1482	Rat	5th	"	24.7	30.0	21.0
" 13	1482	"	9th	"	23.5	31.5	19.0
" 16	1643	"	8th	"	19.6	21.0	18.0
					23.6	34.0	15.0

* B, vol. 81, 1909, pp. 16 and 17.

It will be seen from the above table that *Trypanosoma brucei*, Uganda strain, 1909, varies in length between 15 and 34 microns. Doubtless, individuals could be found slightly longer or shorter than this if they were hunted for. In Table 48, as already mentioned, the 20 trypanosomes drawn are the first met with, except that dividing forms are left out, and this rule is adhered to throughout.

The average length of the 172 trypanosomes dealt with in Table 48 is 23·6 microns; but, as this does not give much information, the following table (49) has been prepared. This represents the length of each of 160 trypanosomes, in eight preparations of 20 each. Although *Trypanosoma brucei* is described as a dimorphic species, it must not be supposed that there is any strict line of demarcation between the long and slender and short and stumpy forms. There are the intermediate forms. This table is also useful in showing at a glance the distribution of the various lengths.

Table 49.—Represents the distribution in respect to length of 160 individuals of *Trypanosoma brucei*, Uganda strain, 1909.

Experiment No.	Short and Stumpy.							Inter-mediate.			Long and Slender.														Average, in microns.
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.					
1479	—	—	—	—	—	—	—	3	—	1	2	4	2	4	1	2	—	1	—	—	—	26·5			
1479	—	1	1	—	1	3	2	2	4	3	—	—	—	1	2	—	—	—	—	—	—	22·0			
1479	—	—	—	—	—	4	4	4	—	1	3	—	—	—	—	1	—	—	—	2	1	23·5			
1644	1	1	2	4	1	1	3	2	2	1	—	—	—	—	1	—	—	—	—	—	1	20·7			
1644	—	—	1	—	1	1	—	—	—	—	3	1	2	1	4	3	1	—	—	1	1	27·1			
1482	—	—	—	—	—	—	1	2	5	3	3	1	1	2	1	1	—	—	—	—	—	24·7			
1482	—	—	—	—	3	4	3	1	1	—	1	—	—	1	3	2	—	—	1	—	—	23·5			
1643	—	—	—	2	4	13	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19·6			
Totals ...	1	2	4	6	10	26	14	14	12	9	12	6	6	12	10	7	1	2	3	3	—				
Percentages...	0·6	1·3	2·5	3·8	6·2	16·2	8·7	8·7	7·5	5·6	7·5	3·8	3·8	7·5	6·2	4·4	0·6	1·3	1·9	1·9	—				

This division of *Trypanosoma brucei* into long, intermediate, and short forms is, of course, quite artificial, as the one form passes into the other by insensible gradations. It may, however, prove of some use in the identification of this species. In the same way a series of measurements of the various parts of the trypanosome body may also prove useful. The following table gives the distance between the posterior extremity and the micronucleus, the micronucleus and the nucleus, the length of the nucleus, the distance between the nucleus and the anterior extremity, and the length of the free flagellum (*see* p. 145).

Breadth.—The long and slender forms average 1·5 micron; the short and stumpy 2·5 microns.

Shape.—As already mentioned, this is a markedly dimorphic species, composed of long, slender forms with free flagella, and short, stout forms without free flagella. It is curious that the long forms may preponderate in the blood one day and the short forms another. For example, as will be seen from Table 48, the trypanosomes in the guinea-pig's blood on October 11 were mostly short forms, giving an average of 20·7 microns, while a week later they were mostly long forms, giving an average of 27·1 microns. Note also Rat 1643, with an average of 19·6 microns, and Rat 1482, with an average of 24·7 microns.

This species may be compared with Dutton and Todd's *Trypanosoma dimorphon*, and in truth the coloured drawings given by them in their original plate* (figs. VII, IX, and X) are very similar, if not identical, in shape and size with *Trypanosoma brucei*. Fig. X, the long, slender form with free flagellum, is 30·5 microns in length. Fig. VII, the short, stumpy form without free flagellum, is 16 microns in length. As to fig. VI, on the same plate, stated to represent the "tadpole form," one would suspect from its general shape and appearance that it had no connection with the others. But in regard to figs. VII and X, anyone comparing Dutton and Todd's plate with the coloured plate accompanying this paper must be struck with the close resemblance between these two forms of *Trypanosoma dimorphon* and *Trypanosoma brucei*. Is it possible that Dutton and Todd were dealing with *Trypanosoma brucei* when they described *Trypanosoma dimorphon*?

Contents of Cell.—The protoplasm which is stained a pale blue is often dotted over with chromatin granules, especially in the anterior half (Plate 5). In well-stained preparations the distribution and number of these granules is sufficient, according to McFadyean, to differentiate this species from *Trypanosoma evansi*. This was also pointed out by Laveran and Mesnil some years ago, when they wrote: "Avec un peu d'habitude on arrive au contraire à distinguer les deux Trypanosomes, quand on dispose de préparations bien colorées et riches en parasites."†

Nucleus.—Is oval or elongated in shape in the long forms, a short oval in the intermediate, round or oval in the short, stumpy forms, and situated about the middle of the body.

Micronucleus.—Small and round, situated, on an average, 2·1 microns from the posterior extremity in the long and slender forms, 1·4 microns in the intermediate, and 1·1 microns in the short and stumpy.

Undulating Membrane.—This is well developed in this species and thrown into many folds and undulations. In this it differs markedly from *Trypanosoma vivax*.

Flagellum.—The flagellum in the long and slender forms is free and, on an average, 6·4 microns in length. In the short and stumpy forms there is no free flagellum.

* 'First Report of the Trypanosomiasis Expedition to Senegambia (1902),' University Press of Liverpool, 1903.

† 'Trypanosomes et Trypanosomiasés,' 1904.

Table 50.—Measurements of the various Parts of *Trypanosoma brucei*, Uganda strain, 1909.

Experi- ment.	Posterior extremity to micro- nucleus.	Micro- nucleus to nucleus.	Length of nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
LONG AND SLENDER FORMS.						
1644	4	5	3.5	11.5	10	34
1644	3	7	4	10	10	34
1644	3	6.5	3.5	13	7	33
1644	2.5	7.5	3	10	8	31
1644	2	7	3.5	12.5	5	30
1644	2	7	3	11	7	30
1644	2	7	3	8	10	30
1482	1	7	3	11	8	30
1627	2	6	2.5	10.5	9	30
1627	3	7.5	2.5	13	4	30
1644	2	6	3	12	6	29
1644	3.5	5.5	3.5	9.5	7	29
1644	2	6.5	3	12.5	5	29
1644	2.5	7	3	6.5	10	29
1482	2	6	3.5	9.5	8	29
1644	3	6	3	8	8	28
1644	2	7	3	16	—	28
1482	1.5	6.5	2.5	13.5	4	28
1482	1.5	8	2.5	8	8	28
1627	1	7.5	2.5	11	6	28
1627	1	7.5	2.5	8	9	28
1644	2	7	3	10	5	27
1644	2	6	3.5	8.5	7	27
1627	1	7	3	9	7	27
1482	2	7.5	2	11.5	4	27
1644	2	6	2.5	10.5	5	26
1482	2	7	3	9.5	4.5	26
1644	2	6	3	7	7	25
1644	2	7	3.5	8.5	4	25
1644	1.5	5.5	3	10	5	25
1482	2	5.5	2.5	10	5	25
1482	1	6	3	11	4	25
1482	2	6.5	3	8.5	5	25
1627	2	6	2.5	8.5	6	25
Average	2.1	6.6	3.0	10.2	6.4	—
INTERMEDIATE FORMS.						
1644	1	6	2.5	14.5	—	24
1482	2	6.5	3.5	8	4	24
1482	1	6	3	10	4	24
1482	1.5	6	3	9.5	4	24
1644	1	5	2.5	14.5	—	23
1644	1	7.5	2.5	12	—	23
1482	1	6	3	8.5	4.5	23
1482	2	5	2.5	8.5	5	23
1482	2	5	4	9	3	23
1482	1	5.5	3	9	4.5	23
1482	2	5	3	10	3	23
1627	1.5	7	3.5	4	7	23
1644	2	6.5	3	5.5	5	22
1944	1	7	3.5	6.5	4	22
1482	2	5.5	2	12.5	—	22
1482	1.5	6	2.5	7	5	22
1627	1	6	2	7	6	22
Average	1.4	6.0	2.9	9.2	3.5	—

Table 50.—Measurements of the various Parts of *Trypanosoma brucei*, Uganda strain, 1909—*continued*.

Experi- ment.	Posterior extremity to micro- nucleus.	Micro- nucleus to nucleus.	Length of nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
SHORT AND STUMPY FORMS						
1644	1	6	3	11	—	21
1644	1	5·5	2·5	12	—	21
1644	1	5·5	2·5	12	—	21
1482	2	5	3	11	—	21
1643	1	4·5	2·5	13	—	21
1627	1·5	6·5	3	7·5	2·5	21
1644	2	3	3	12	—	20
1644	1	5	2	12	—	20
1643	1	4·5	2	12·5	—	20
1643	1	4	2·5	12·5	—	20
1643	1	6	2·5	10·5	—	20
1643	1	5	2·5	11·5	—	20
1643	1	5·5	2	11·5	—	20
1643	1	5	3	11	—	20
1643	1	5·5	2·5	11	—	20
1643	1	4·5	2·5	12	—	20
1643	1	4	2·5	12·5	—	20
1643	1·5	5·5	2	11	—	20
1643	1	4	2·5	12·5	—	20
1643	1	5	2	12	—	20
1643	1	4·5	2	12·5	—	20
1627	1	6	2	11	—	20
1644	1	5	2	11	—	19
1644	1	3·5	2	12·5	—	19
1643	1	5	2·5	10·5	—	19
1643	1	5	2	11	—	19
1643	1	4·5	2	10·5	—	19
1643	1	5·5	3	10·5	—	19
1627	1	7	3	8	—	19
1644	1	4	2·5	10·5	—	18
1644	1	4	2·5	10·5	—	18
1644	1	3·5	3	10·5	—	18
1644	1	6	2·5	8·5	—	18
1643	1	4·5	2·5	10	—	18
1643	2	4	2·5	9·5	—	18
1644	1	5	3	8	—	17
1644	1	4·5	2·5	9	—	17
1644	1	6	2·5	7·5	—	17
1644	1	5·5	2	7·5	—	16
1644	1	5	2	7	—	15
Average ...	1·1	4·9	2·4	10·7	—	—
Average of three forms	1·5	5·8	2·8	10·0	3·3	—

Comparison of Trypanosoma brucei, Uganda, 1909, with Trypanosoma brucei, Zululand, 1894 (see Plate 4).

When *Trypanosoma brucei* was discovered in Zululand, in 1894, it was naturally thought to be the one and only trypanosome in Africa, and a detailed description seemed unnecessary. Now, however, since the number of species in Africa has increased to such an alarming extent, it is necessary to add to the old description by more detailed measurements. Luckily, many of the old Zululand preparations are still extant, so that it has been possible to do this. It must, however, be borne in mind that the Zululand preparations are some 15 years old, and were stained with carbol-fuchsin and not with Giemsa as in the case of the Uganda strain. They may, therefore, have shrunk to a greater extent than the more recently stained specimens.

Table 51.—Measurements of the Original Strain of *Trypanosoma brucei*, discovered in Zululand in 1894.

Trypanosoma brucei, Zululand, 1894.

Date.	No. of experiment.	Animal.	Method of fixing and staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1/11/95	212	Horse	Osmic acid ; carbol-fuchsin.	22·4	32·0	14·6
1/11/95	212	"	" "	20·6	32·5	14·0
7/11/95	219	Donkey	" "	23·0	35·0	14·5
23/11/95	214	Ox	" "	18·0	25·5	13·0
4/7/97	—	Monkey	" "	20·5	32·0	15·0
6/1/95	190	Dog	" "	25·0	35·0	17·0
10/12/95	—	"	" "	24·0	32·0	17·0
14/1/96	233	"	" "	27·8	34·0	18·0
25/1/96	244	"	" "	20·6	33·0	15·0
7/2/96	229	"	" "	28·7	35·0	19·0
9/7/97	433	"	" "	19·7	29·5	17·0
				22·8	35·0	13·0

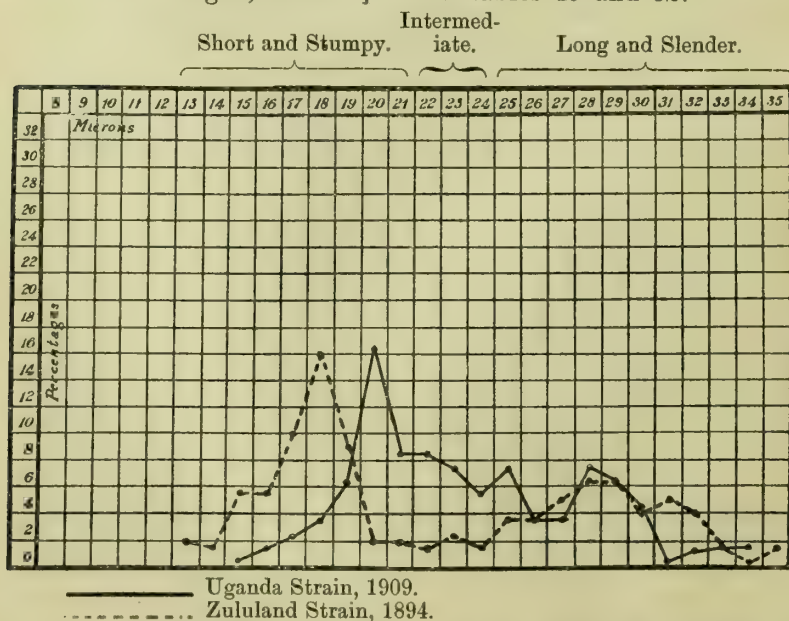
Table 52.—Represents the distribution in respect to length of 200 individuals of *Trypanosoma brucei*, Zululand strain, 1894.

Experiment No.	Short and Stumpy.										Inter- mediate.			Long and Slender.																	Average in microns.	
	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.									
212	—	—	1	2	2	3	1	—	—	1	1	—	—	2	1	1	2	2	—	1	—	—	—	—	—	—	—	—	—	—	—	22.4
212	—	1	3	2	2	4	1	—	—	—	—	—	—	2	2	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	20.6
219	—	—	3	3	1	1	1	—	—	—	—	—	—	1	1	2	2	3	—	1	—	—	—	—	—	—	—	—	—	—	—	23.0
214	3	2	1	1	5	1	—	1	3	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18.0
—	1	—	2	2	5	1	2	—	—	—	—	1	1	—	1	1	1	1	—	1	—	—	—	—	—	—	—	—	—	—	—	20.5
—	—	—	—	—	2	3	1	1	1	1	2	—	—	1	1	1	1	2	2	1	—	—	—	—	—	—	—	—	—	—	—	24.0
233	—	—	—	—	—	2	1	—	—	—	—	1	—	—	—	3	2	1	1	3	2	2	1	—	—	—	—	—	—	—	—	27.8
244	—	—	1	1	—	7	5	2	—	—	—	—	—	—	—	—	1	1	1	—	—	1	—	—	—	—	—	—	—	—	—	20.6
229	—	—	—	—	—	—	2	—	—	—	—	—	1	1	—	—	—	4	3	1	4	2	—	—	—	—	—	—	—	—	—	28.7
433	—	—	—	—	3	10	3	—	—	—	—	—	1	—	1	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	19.7
Totals	...	4	3	11	11	20	32	17	4	4	3	5	3	7	7	10	13	13	8	10	8	3	1	3	—	—	—	—	—	—	—	—
Percentages...		2	1.5	5.5	5.5	10	16	8.5	2	2	1.5	2.5	1.5	3.5	3.5	5	6.5	6.5	4	5	4	1.5	0.5	1.5	—	—	—	—	—	—	—	—

The average length of the *Trypanosoma brucei*, Uganda, 1909, was 23.6 microns, the maximum 34 microns, and the minimum 15 microns. The measurements of the two strains therefore correspond very closely.

Tables 49 and 52 can be compared in the following chart, which gives the curves of the distribution, in respect to length, of *Trypanosoma brucei*, Uganda, 1909, and *Trypanosoma brucei*, Zululand, 1894.

Chart giving Curves of the Distribution, by percentages, in respect to Length, made up from Tables 49 and 52.



From these curves it will be seen that there is a marked resemblance between the two strains when represented in this way.

Animals Susceptible to Trypanosoma brucei.

Table 53 shows the effect of inoculating various animals with *Trypanosoma brucei*, Uganda strain, 1909.

The number of experiments carried out on the various animals is too small for comparative purposes, but sufficient to show that cattle and the smaller laboratory animals are susceptible to this trypanosome.

Table 53.—Animals susceptible to *Trypanosoma brucei*, Uganda, 1909.

Date.	Experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
CATTLE.					
1909. Aug. 11	1461	Nat. infec.	?	—	Killed on 79th day.
GOAT.					
Aug. 16	1478	Ox 1461	—	—	Never showed trypanosomes; under observation 37 days.
MONKEY.					
Aug. 16	1480	Ox 1461	—	—	Died 10 days after inoculation; never showed trypanosomes.
Sept. 4	1627	Dog 1479	13	69	Died of <i>T. brucei</i> .
DOG.					
Aug. 16	1479	Ox 1461	8	49	Died of <i>T. brucei</i> .
RABBIT.					
Sept. 8	1645	Rat 1482	26	—	Still alive after 67 days.
GUINEA-PIG.					
Aug. 16	1481	Ox 1461	—	—	Never showed trypanosomes; under observation 18 days.
Sept. 3	1481	Dog 1479	5	—	Still alive after 71 days.
" 8	1644	Rat 1482	26	—	" " 67 "
RAT.					
Aug. 16	1482	Ox 1461	—	—	Never showed trypanosomes; under observation 18 days.
Sept. 4	1482	Dog 1479	5	23	Died of <i>T. brucei</i> .
" 8	1643	Rat 1482	8	22	" "
MOUSE.					
Aug. 16	1483	Ox 1461	—	—	Accidentally killed on 9th day; never showed trypanosomes.

Cultivation of Trypanosoma brucei, Uganda strain, 1909.

No attempt was made in Uganda to cultivate this trypanosome.

Carrier of Trypanosoma brucei.

No experiments were made in the laboratory at Mpumu with *Glossina palpalis* as a carrier of this trypanosome, and no evidence is to hand as to how it is conveyed from sick to healthy animals in the district of Bukedi.*

Conclusion.

With the evidence available, the Commission consider themselves justified in considering the trypanosome recovered from the Uganda ox to be identical with *Trypanosoma brucei*, the cause of Nagana in Zululand and other parts of South Africa.

DESCRIPTION OF PLATES.

PLATE 4.

- (1) *Trypanosoma brucei*, Zululand, 1894, stained carbol-fuchsin. × 2000.

FIGS. 1 and 2.—Long and slender forms.

FIGS. 3 and 4.—Intermediate forms.

FIGS. 5, 6 and 7.—Short and stumpy forms.

- (2) *Trypanosoma brucei*, Uganda, 1909, stained Giemsa. × 2000.

FIGS. 8 and 9.—Long and slender forms.

FIG. 10.—Intermediate form.

FIGS. 11, 12 and 13.—Short and stumpy forms.

- (3) *Trypanosoma dimorphon* (?), Khartoum. × 2000.

FIGS. 14 and 15.—Long and slender forms.

FIG. 16.—Intermediate form.

FIGS. 17, 18 and 19.—Short and stumpy forms.

PLATE 5.

Trypanosoma brucei, Uganda, 1909, stained Giemsa. × 2000.

FIGS. 1-5.—Long and slender forms.

FIGS. 6-10.—Intermediate forms.

FIGS. 11-16.—Short and stumpy forms.

28.—TRYPANOSOMA VIVAX (ZIEMANN).†

Synonyms.

Trypanosome of Pordage's ox, 1903 (Sleeping Sickness Commission of the Royal Society, 1903).

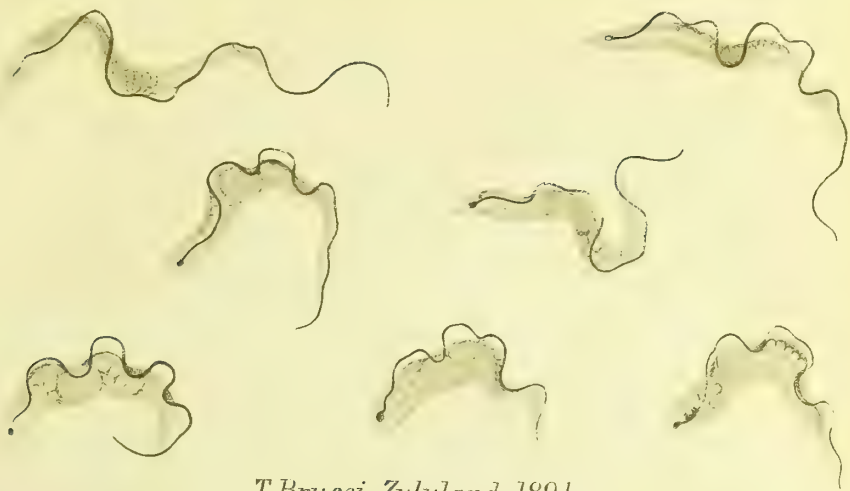
Trypanosoma cazalbouii (souma). Laveran.

Introduction.

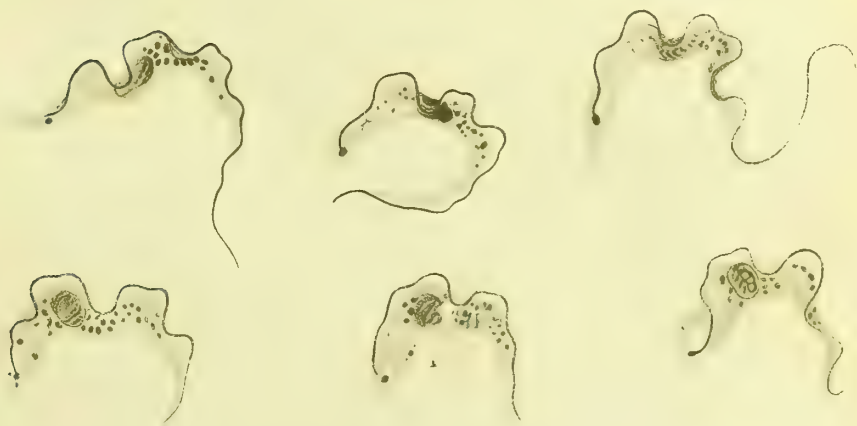
This interesting species of trypanosome appears to be widely distributed in Uganda. It was first discovered by the Commission in two cattle which came from Kavirondo, the district lying to the

* Since this paper was written a few experiments have been carried out (see p. 32 of this Report).

† Reprinted from the 'Proceedings' of the Royal Society, B, vol. 83, 1910.



T. Brucei. Zululand, 1894.



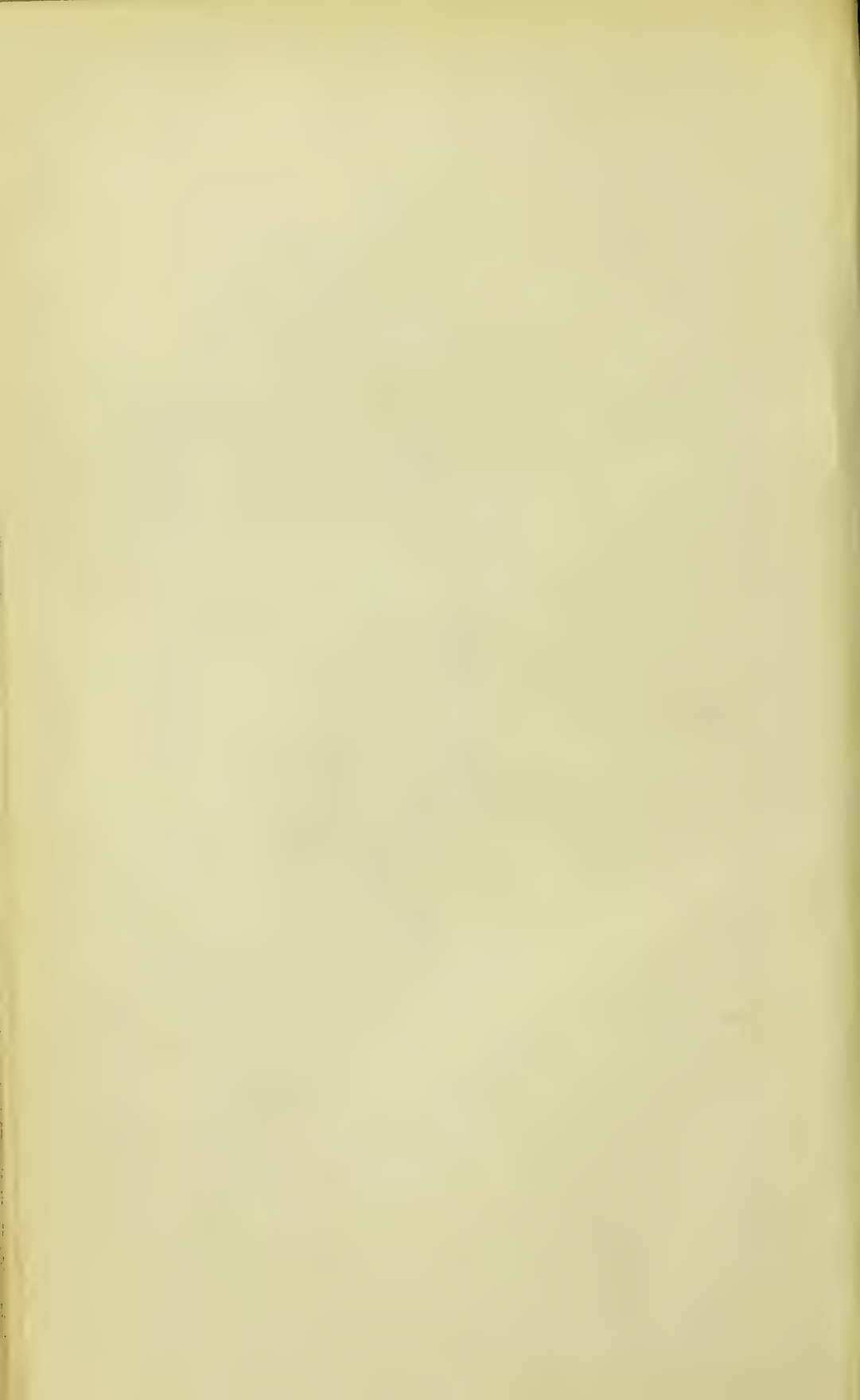
T. Brucei, Uganda, 1909.

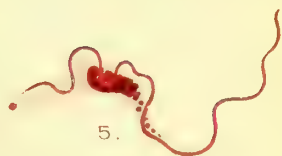


M. E. Bruce, del.

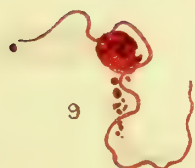
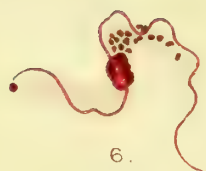
T. Dimorphon ? Khartoum.

Pl. 4. 1894.

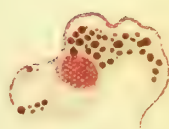
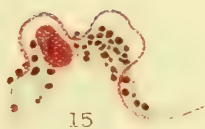
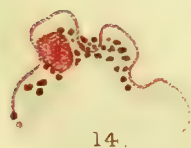
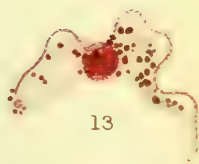
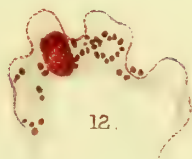
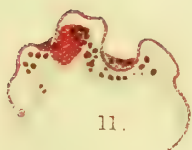




Long & Slender.



Intermediate.



Short & Stumpy.



north-east of Victoria Nyanza. These oxen were driven to Kampala round the north end of the Lake, and probably became infected on the way.

Then the Government Transport Department lost many of their oxen from this trypanosome. They were worked between Kampala, the native capital, and Luzira, the port on the Lake-shore, which lies about seven miles to the south-east. When the epidemic broke out these cattle were kraaled near the Lake-shore, along which they were allowed to graze, and where tsetse-flies are numerous. Afterwards, at the suggestion of the Commission, they were kraaled at Kampala, when the epidemic stopped, and no more deaths from *Trypanosoma vivax* occurred among them.

Cases also occurred among the transport cattle belonging to the German Company, "Victoria Nyanza Agentur." These cattle were employed carrying goods between Lake Victoria and Lake Albert.

Lastly, the *Glossina palpalis* on the Lake-shore near Mpumu, where the Sleeping Sickness Commission laboratory was placed, were found to be naturally infected with *Trypanosoma vivax*, and this trypanosome was found in the blood of a bush-buck, shot at the same place at which the flies were collected.

The evidence, therefore, at present points to the *Glossina palpalis* being the carrier of the disease, and that the wild animals living in the fly-area act as a reservoir of the virus.

This species of trypanosome is similar to *Trypanosoma nanum*, in that it is only pathogenic to equines and bovines, and has no effect on the smaller laboratory animals. There was no opportunity in Uganda of inoculating it into horses, mules, or donkeys.

Nine cattle came under observation suffering from a natural infection of the disease. Seven of these died after being at Mpumu, on an average, 103 days. Five cattle were successfully inoculated, and these died, on an average, in 69 days. The disease is, therefore, a fairly rapid and fatal one in cattle. It may be noted that two of the cattle (Experiments 493 and 583), after living for 268 and 237 days respectively, died at last from an accidental infection of *Trypanosoma pecorum*. It is possible that these two oxen had recovered, and if not, it is certain that trypanosomes were either absent or very scanty in their blood, as inoculations from them failed to infect goats and sheep. It may also be noted that none of the four sheep which were inoculated ever showed signs of the disease. This is probably due to the scarcity or absence of the trypanosomes in the blood used in the experiment for inoculation, and not to any natural immunity.

Morphology of Trypanosoma vivax.

This species of pathogenic trypanosome can at once be recognised among all the others by its shape alone. There is no tendency to dimorphism, as in *Trypanosoma brucei* and *Trypanosoma gambiense*. On the contrary, the individuals tend to run persistently to one type. This type has an average length of 24 microns. The body posterior to the nucleus is swollen, and contains clear protoplasm, in which an alveolar structure can be made out; the posterior end is rounded, as a rule, and close to it

is the large round micronucleus; the anterior part of the body rapidly narrows and terminates in a free flagellum; the nucleus is elongated and situated in the narrowed part, is often broken up and diffused, and seems to fill up the part of the body in which it lies; the undulating membrane is narrow and simple, and can, as a rule, only be made out by the deeply-stained flagellum running along its border. An examination of the plates which illustrate this paper will, however, give a clearer idea of the general appearance of this species than any written description.

(a.) *Living, Unstained.*

This species of trypanosome is extremely active in its movements. It dashes across the field of the microscope with such rapidity that it is impossible to follow its movements, cyclone-like leaving a clear path, the corpuscles in its track having been flung on either side. If it remains at the same spot for a time, as it sometimes does, it has an appearance of great energy and power, throwing the surrounding red blood corpuscles about in wild confusion.

(b.) *Fixed and Stained.*

The blood-films were fixed, stained and measured as described in the 'Proceedings' of the Royal Society.*

Length.—The following table gives the average length of this trypanosome in the ox and goat. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given.

Table 54.—Measurements of *Trypanosoma vivax*, Uganda, 1909.

No. of experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
290	Ox	Osmic acid	Giemsa	23·7	28·0	21·0
445	"	"	"	23·8	26·0	20·0
450	"	"	"	24·4	27·0	22·0
450	"	"	"	23·4	25·0	22·0
493	"	"	"	25·0	27·0	22·0
597	"	"	"	25·1	28·0	22·0
598	"	"	"	25·8	28·0	24·0
779	"	"	"	24·6	27·0	21·0
982	"	"	"	23·5	25·0	22·0
1267	"	"	"	24·2	27·0	20·0
1462	"	"	"	23·8	28·0	16·0
864	Goat	"	"	24·2	25·0	23·0
864	"	"	"	24·7	26·0	19·0
1036	"	"	"	23·5	29·0	19·0
				24·2	29·0	16·0

As is shown by the above table, this trypanosome varies in length between 16 and 29 microns. Individuals only 16 microns

* B, vol. 81, 1909, pp. 16, 17.

long are rare, and only occur immediately after division. The following table represents the distribution of length among 180 trypanosomes, and it will be seen by it that only three are found to measure less than 19 microns.

Table 55.—Represents the Distribution in Respect to Length of 180 Individuals of *Trypanosoma vivax*, Uganda Strain, 1909.

Experiment.	Microns.														Average, in microns.
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	
445	—	—	—	—	1	—	2	3	7	6	1	—	—	—	23·8
450	—	—	—	—	—	—	1	4	8	—	6	—	—	—	24·4
597	—	—	—	—	—	—	1	1	3	9	3	—	1	—	25·1
598	—	—	—	—	—	—	—	—	4	1	11	3	1	—	25·8
779	—	—	—	—	—	1	2	2	2	7	3	3	—	—	24·6
1267	—	—	—	—	1	1	—	5	3	4	5	1	—	—	24·2
1462	1	2	—	—	—	—	1	1	5	3	4	2	1	—	23·8
864	—	—	—	1	—	—	—	2	2	9	6	—	—	—	24·7
1036	—	—	—	1	2	1	4	3	1	5	1	—	—	2	23·5
Totals ...	1	2	—	2	4	3	11	21	35	44	40	12	3	2	—
Percentages	0·6	1·1	—	1·1	2·2	1·7	6·1	11·6	19·5	24·4	22·2	6·7	1·7	1·1	—

Breadth.—Varies between 2 and 3 microns.

Shape.—This species can be recognised with certainty by its shape alone. The body of the creature lies mostly posterior to the nucleus, and this part is broad and swollen and filled with beautifully clear protoplasm, in which a delicate alveolar structure can be made out. The body narrows at the nucleus and tapers off rapidly to the anterior extremity (Plates 6 and 7).

Contents of Cell.—Clear, with a delicate alveolar structure, and now and then a chromatin-staining granule, especially in the narrow anterior part.

Nucleus.—Long and oval, often diffuse, situated towards the anterior extremity, and in a narrowed or waist-like part of the trypanosome.

Micronucleus.—Large, round and terminal, or sub-terminal.

Undulating Membrane.—Narrow, simple, straight, and little in evidence.

Flagellum.—There is a well-marked flagellum, the free part varying from 3 to 6 microns in length (Plates 6 and 7).

Comparison of Trypanosoma vivax, Uganda, 1909, with Trypanosoma vivax, Cameroons, 1903.

Thanks to the courtesy of the Director of the Hamburg Institute of Tropical Medicine, through Dr. Fülleborn, the Commission are enabled to compare the Uganda strain with the original preparations made by Ziemann in 1903.

Table 56.—Measurements of the Original Strain of *Trypanosoma vivax* (Ziemann), 1903.

Animal.	Method of fixing and staining.	In microns.		
		Average length.	Maximum length.	Minimum length.
Ox	?	24·8	26·0	23·0
"	?	26·5	31·0	23·0
Sheep	?	21·4	23·0	18·0
"	?	22·1	25·0	20·0
		23·7	31·0	18·0

The trypanosomes found in the blood of this sheep were shorter, narrower, and have a more pointed posterior extremity than in the type described. Whether this is the rule or only exceptional it is impossible with our scanty material to say.

Table 57.—Represents the Distribution in Respect to Length of 80 Individuals of *Trypanosoma vivax*, Cameroons Strain, 1903.

Experiment.	Microns.															Average. in microns.
	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.		
Ox	—	—	—	—	—	3	4	7	6	—	—	—	—	—	24·8	
...	—	—	—	—	—	1	1	3	3	9	2	—	—	1	26·5	
Sheep	1	2	1	5	6	5	—	—	—	—	—	—	—	—	21·4	
"	—	—	4	3	4	5	3	1	—	—	—	—	—	—	22·1	
Totals	1	2	5	8	10	14	8	11	9	9	2	—	—	—	—	
Percentages	1·2	2·5	6·3	10·0	12·5	17·5	10·0	13·7	11·3	11·3	2·5	—	—	1·2	—	

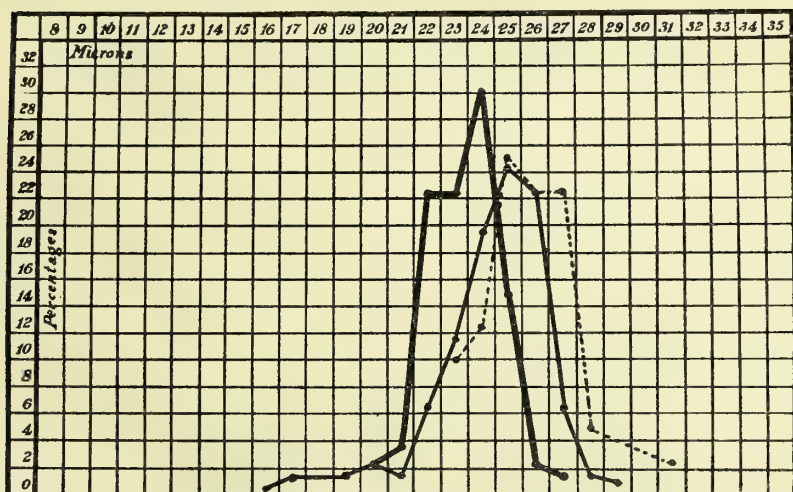
Comparison of Trypanosoma vivax, Uganda, 1909, with Trypanosoma vivax, 1903 (Pordage's Ox).

On examining the preparations made in 1903 from two oxen belonging to the Uganda Transport, the trypanosomes in them were at once recognised as belonging to this species (Plate 6).

Table 58.—Measurements of *Trypanosoma vivax*, Uganda, 1903 (Pordage's Ox).

No. of experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1	Ox	Osmic acid	Leishman	23·3	25·0	21·0
1	"	"	"	23·1	26·0	20·0
2	"	"	"	23·3	25·0	22·0
2	"	"	"	23·5	27·0	20·0
				23·3	27·0	20·0

Chart giving curves representing the distribution, by percentages, in respect to length, of *Trypanosoma vivax*, Uganda, 1909, *Trypanosoma vivax*, Uganda, 1903 (Pordage's ox), and *Trypanosoma vivax*, Cameroons, 1903.



- *T. vivax*, Uganda, 1909 ; 180 trypanosomes from oxen and goats.
 — *T. vivax*, Uganda, 1903 (Pordage's ox) ; 80 trypanosomes from ox.
 - - - *T. vivax*, Cameroons, 1903 ; 40 trypanosomes from ox.

Table 59.—Represents the Distribution in Respect to Length of 80 Individuals of *Trypanosoma vivax*, Uganda, 1903 (Pordage's Ox).

Experiment.	Microns.								Average, in microns.
	20.	21.	22.	23.	24.	25.	26.	27.	
1	—	1	4	6	5	4	—	—	23.3
1	1	—	6	5	5	2	1	—	23.1
2	—	—	6	4	7	3	—	—	23.3
2	1	2	2	3	7	3	1	1	23.5
Totals ...	2	3	18	18	24	12	2	1	—
Percentages	2.5	3.7	22.5	22.5	30.0	15.0	2.5	1.3	—

From a comparison of these tables and the figures on Plate 6, there can be little doubt that the trypanosome found in the blood of cattle in Uganda in 1909 is identical with that seen in 1903, and also with that discovered by Ziemann in the Cameroons, and named by him *Trypanosoma vivax*. As Dr. Laveran kindly examined the Uganda specimens and pronounced them to be *Trypanosoma cazalboui*, it is probable that *Trypanosoma vivax* and *Trypanosoma cazalboui* are the same species.

Laveran
determines them

Animals Susceptible to Trypanosoma vivax, Uganda, 1909.

Table 60.

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
CATTLE.					
1909.					
July 12	431	Nat. infec.	?	—	Alive after 38 days.
Jan. 14	450	"	?	48	Died of <i>T. vivax</i> .
Feb. 2	493	"	?	268	Also infected by <i>T. pecorum</i> .
Mar. 6	583	"	?	237	"
" 14	597	"	?	49	Died of <i>T. vivax</i> .
" 14	598	"	?	79	" "
May 16	779	"	?	6	" "
July 12	1309	"	?	40	" "
" 12	1318	"	?	—	Alive after 30 days.
Sept. 29	290	Ox 1465	30	32	Died of <i>T. vivax</i> .
Mar. 3	467	Ox 450	—	—	Experiment stopped after 77 days.
May 19	869	Oxen 493, 583 & 598.	—	—	Experiment stopped after 101 days.
" 19	870	"	—	—	Experiment stopped after 101 days.
July 27	737	Goat 864	15	60	Died of <i>T. vivax</i> .
June 21	1030	"	10	83	" "
July 6	1267	"	22	55	" "
" 6	1268	"	15	114	Killed.
		Average ...	18	89	
GOAT.					
1909.					
Mar. 31	623	Ox 598	—	—	Experiment stopped after 48 days.
Apr. 2	636	Ox 494	—	—	Experiment stopped after 46 days.
" 3	639	Ox 583	—	—	Experiment stopped after 45 days.
May 19	864	Ox 779	15	148	Died of <i>T. vivax</i> .
Aug. 4	1419	Ox 1309	22	—	Still alive after 30 days.
Sept. 10	1652	Ox 1318	21	31	Died of <i>T. vivax</i> .
June 22	1036	Goat 864	27	29	" "
—	1037	"	—	—	Never showed trypanosomes; under observation 66 days.
June 25	1079	"	21	—	Experiment stopped after 21 days.
July 19	1344	"	36	—	Experiment stopped after 36 days.
" 26	1383	"	24	—	Experiment stopped after 24 days.
Aug. 6	1433	"	12	17	Died of <i>T. vivax</i> .
		Average ...	22	56	

* Duration includes the days of incubation; it dates from the day of infection.

Animals Susceptible to Trypanosoma vivax, Uganda, 1909.

Table 60—continued.

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
SHEEP.					
1909.					
Mar. 23	610	Ox 598	—	—	Experiment stopped after 56 days.
Apr. 2	637	Ox 493	—	—	Experiment stopped after 46 days.
May 19	863	Ox 779	—	—	Died 124 days after inoculation.
June 17	1013	Ox 583	—	—	Experiment stopped after 72 days.
MONKEY.					
Feb. 4	487	Ox 450	—	—	Experiment stopped after 57 days.
„ 4	488	„	—	—	Experiment stopped after 57 days.
Apr. 3	640	Ox 583	—	—	Experiment stopped after 45 days.
„ 3	643	Ox 493	—	—	Experiment stopped after 45 days.
May 19	859	Ox 779	—	—	Experiment stopped after 40 days.
July 9	1304	Ox 1030	—	—	Died 30 days after inoculation.
„ 26	1384	Goat 864	—	—	Experiment stopped after 33 days.
DOG.					
Feb. 4	489	Ox 450	—	—	Died 30 days after inoculation.
Mar. 3	576	„	—	—	Died 31 days after inoculation.
June 18	1021	Ox 583	—	—	Died 28 days after inoculation.
„ 30	1197	Goat 864	—	—	Experiment stopped after 43 days.
July 16	1343	Dog 1021	—	—	Experiment stopped after 31 days.
GUINEA-PIG.					
Mar. 27	617	Ox 598	—	—	Experiment stopped after 52 days.
Apr. 3	642	Ox 583	—	—	Experiment stopped after 45 days.
„ 3	645	Ox 493	—	—	Experiment stopped after 45 days.
May 19	862	Ox 779	—	—	Experiment stopped after 40 days.

* Duration includes the days of incubation ; it dates from the day of infection.

*Animals Susceptible to Trypanosoma vivax, Uganda, 1909.*Table 60—*continued.*

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
RAT.					
1909.					
Mar. 23	609	Ox 598	—	—	Experiment stopped after 56 days.
Apr. 3	641	Ox 583	—	—	Experiment stopped after 45 days.
„ 3	644	Ox 493	—	—	Experiment stopped after 45 days.
May 19	860	Ox 779	—	—	Experiment stopped after 40 days.
MOUSE.					
Mar. 27	616	Ox 598	—	—	Experiment stopped after 52 days.
Apr. 3	646	Ox 583	—	—	Experiment stopped after 45 days.
„ 3	647	Ox 493	—	—	Experiment stopped after 45 days.
May 19	861	Ox 779	—	—	Experiment stopped after 40 days.

* Duration includes the days of incubation ; it dates from the day of infection.

*Cultivation of Trypanosoma vivax.**(a.) Living, Unstained.*

This species grew readily on blood-agar, the blood used in the preparation of the medium being that of the goat. The history of a tube which had been inoculated with a drop of blood from the heart of an ox, Experiment 450, dead of *Trypanosoma vivax* disease, is as follows:—

March 3, 1909.—Inoculated six tubes. The trypanosomes are very scarce in the blood.

March 6.—Tube No. 1 has been examined daily for signs of growth. Nothing living has been seen. The tube remains sterile.

March 8.—To-day many very active trypanosomes are seen. They are single or in small groups of ten or twenty or so. The cell-contents are granular. The cells possess flagella, but no obvious undulating membranes. There are many dividing forms to be seen, and division is evidently going on rapidly, as many individual trypanosomes appear to be undergoing fission into three or more at the same time.

March 9.—Since yesterday an immense multiplication has taken place. The trypanosomes are still very active. They appear to have free flagella and long-pointed, finely granular posterior extremities.

March 11.—Many highly active trypanosomes seen. They are elongated, thin, and have a long free flagellum, sometimes as long as the body itself.

The trypanosomes remained alive and active up to 20 or 30 days, when the tubes became contaminated, or dried up, and were thrown away.

Size.—Cultural forms of *Trypanosoma vivax* vary much in size. At first when they begin to multiply, on or about the sixth day, the individuals forming groups are about 15 microns in length. Next day they are longer and thinner, and later many are seen 50, 60, or more, microns in length.

Shape.—At first, the young forms are oval or elliptical in shape; afterwards they become more attenuated; and later still may be seen as huge trypanosomes with undulating membranes and long free flagella.

Contents of Cell.—In the living unstained condition this appears to be finely granular.

Undulating Membrane.—In the smaller forms composing the groups no obvious undulating membrane can be made out.

Flagellum.—This is free and well developed.

Motion.—The single forms are extremely active.

(b.) *Fixed and Stained* (Plate 8).

Protoplasm.—Is homogeneous, but contains many irregular granules and vacuoles.

Nucleus.—Is usually broken up and diffuse.

Micronucleus.—Often difficult to distinguish. Sometimes placed anterior, at the side of, or posterior to, the nucleus.

Flagellum.—Well-developed free flagellum.

Undulating Membrane.—Present in the older free forms.

The Carrier of Trypanosoma vivax.

It was pointed out in a previous paper that, in laboratory experiments, *Trypanosoma vivax* readily develops in *Glossina palpalis*, and that this tsetse fly can convey the infection to healthy animals after a period of from 20 to 30 days.* It was also found that this fly is naturally infected with this trypanosome, and several animals became ill and died of this disease when freshly-caught Lake-shore flies were fed upon them.† The epidemic among the Government Transport oxen at Kampala also points to this fly being the carrier. As stated above, as long as the cattle were kraaled at the Lake-shore and exposed to the bites of *Glossina palpalis*, so long did they suffer from *Trypano-*

* 'Proceedings' of the Royal Society, B, vol. 82, 1910, p. 381, and p. 26 of this Report.

† 'Proceedings' of the Royal Society, B, vol. 82, 1910, p. 63, and p. 175 of this Report.

soma vivax infection. Afterwards, when stabled at Kampala, the fatality from the disease ceased.

Although it must be confessed the evidence is rather scanty, what there is points to the *Glossina palpalis* as being a carrier, if not the chief carrier, of *Trypanosoma vivax*; and there is no evidence at present to incriminate the *Tabanidae* or *Stomoxys*.

Conclusions.

1. *Trypanosoma vivax*, an easily recognisable species, gives rise to a fatal disease of cattle in Uganda.
2. The carrier of *Trypanosoma vivax* is probably *Glossina palpalis*, which is found naturally infected on the Lake-shore.
3. The reservoir of the virus is possibly the antelope which frequent the *Glossina palpalis* area.

DESCRIPTION OF PLATES.

PLATE 6.

Figures for the comparison of *Trypanosoma vivax*, Uganda, 1909, *Trypanosoma vivax*, Uganda, 1903 (Pordage's ox), and *Trypanosoma vivax*, Cameroons, 1903. $\times 2000$.

PLATE 7.

Trypanosoma vivax, fixed osmic acid, stained Giemsa. $\times 2000$. Note the large round terminal micronucleus; the broad, swollen anterior part filled with clear protoplasm, in which a hint of the alveolar structure is given; the nucleus situated in the narrowed part of the body; the slightly developed undulating membrane; and the free flagellum.

PLATE 8.

FIG. 1.—A group of *Trypanosoma vivax* after 5 days' growth in blood-agar. Stained Giemsa. $\times 2000$.

FIG. 2.—*Trypanosoma vivax* after 6 days' growth. Stained Giemsa. $\times 2000$.

FIGS. 3 and 4.—After 7 days' growth. Stained Giemsa. $\times 2000$.

FIGS. 5-9.—After 8 days' growth. Stained Giemsa. $\times 2000$.

29.—TRYPANOSOMA UNIFORME, SP. NOV.*

Introduction.

The name *Trypanosoma uniforme* has been given to this species on account of the uniformity in shape and general appearance which characterises it.

Only four oxen were found by the Commission to show this trypanosome in their blood. One was received from Sir Apolo Kagwa, K.C.M.G., the Prime Minister, on August 30, 1909, and its blood at once inoculated into a series of animals. The other three came from the Uganda Company's estate at Namukekera.

The ox, goat, and sheep were found to be susceptible, while the monkey, dog, guinea-pig, rat, and mouse proved refractory.

* Reprinted from the 'Proceedings' of the Royal Society, B, vol. 83, 1911.

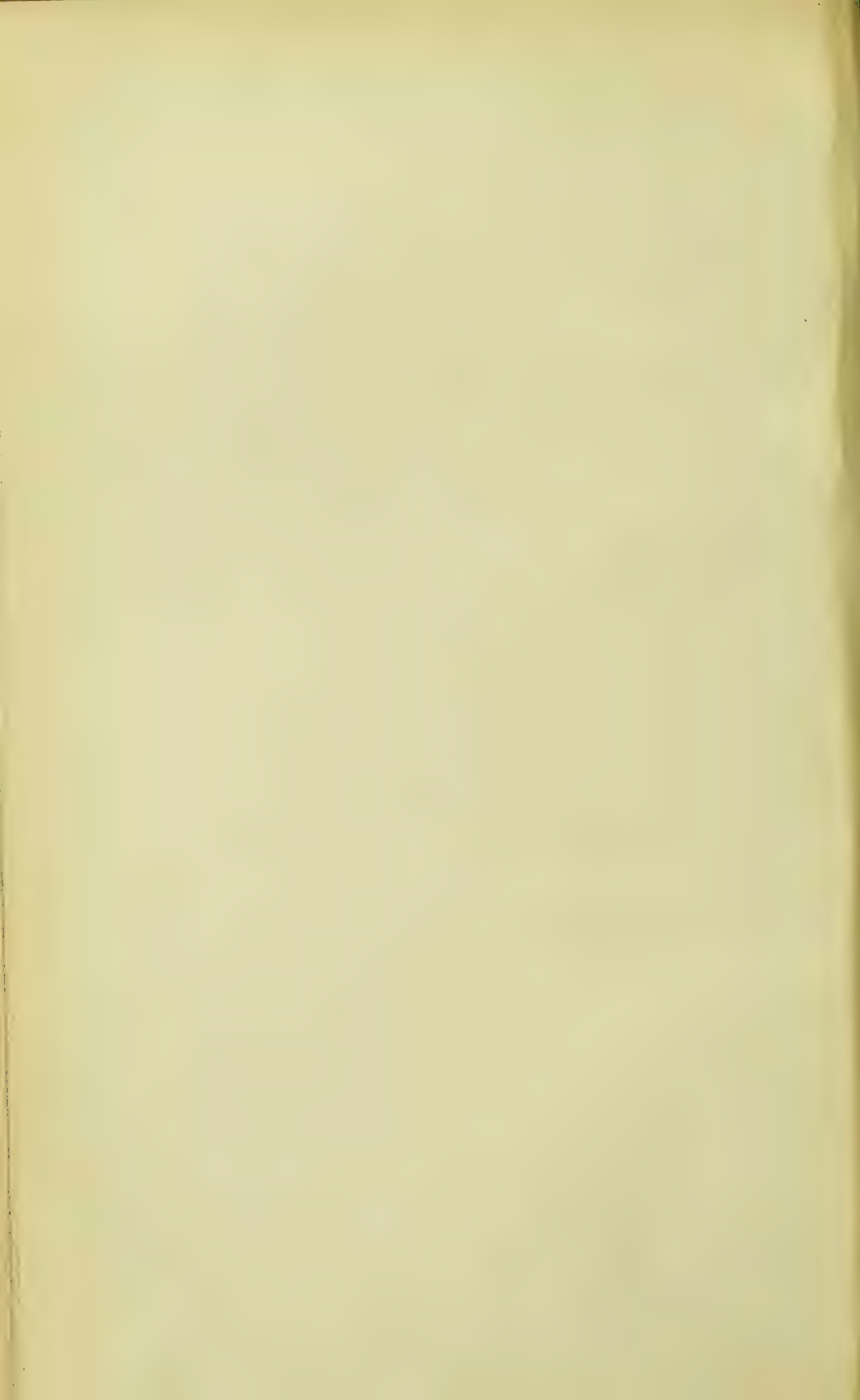


T. Vivax. Uganda, 1909.



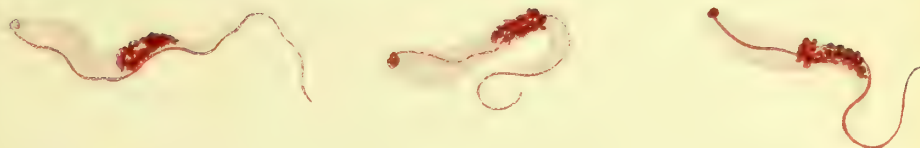
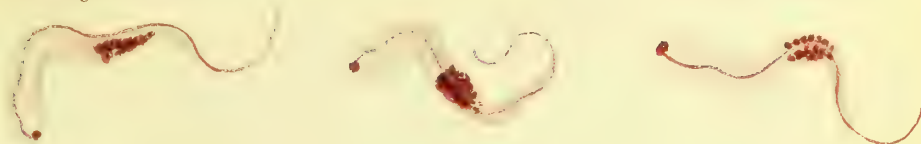
T. Vivax. Portage's Ox, 1903.



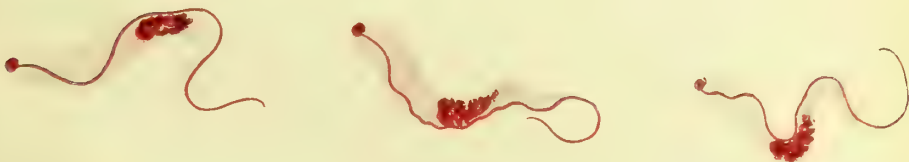
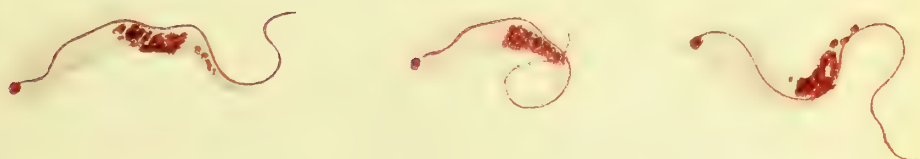


Sleeping Sickness

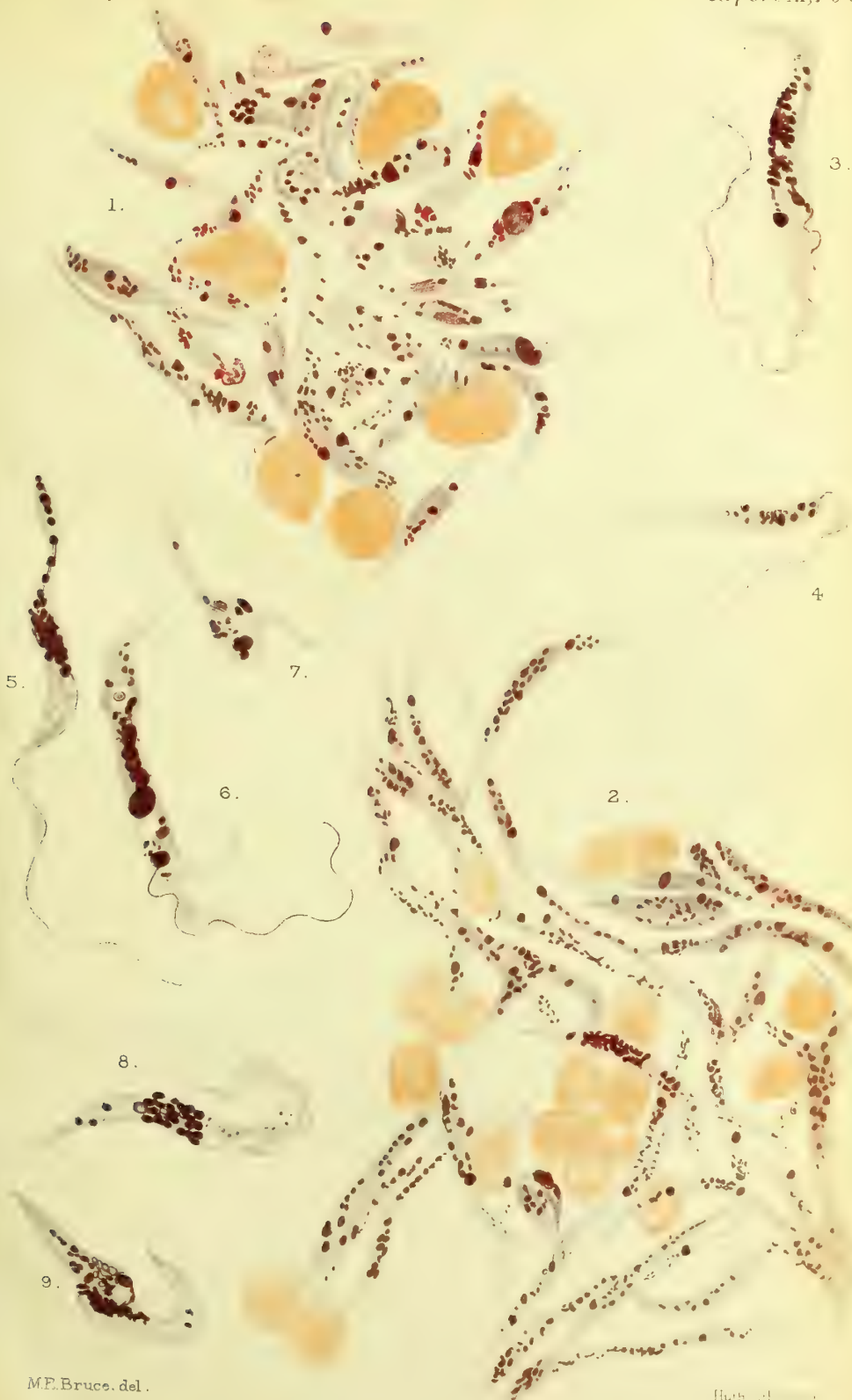
Report XI, Pl. 7.



T. Vivax. Ox.



T. Vivax. Goat.



This was the case with *Trypanosoma vivax*, and, in truth, these two species resemble one another very closely. *Trypanosoma uniforme* differs from *Trypanosoma vivax* in size, and perhaps to a slight extent in shape; but, from the small amount of material at the disposal of the Commission, it would be rash to generalise.

The few facts gathered will, therefore, be put on record, in order to draw the attention of future workers on Uganda trypanosomes to its presence.

Morphology of Trypanosoma uniforme.

(a.) *Living, Unstained.*

This is a small and active trypanosome, which has a marked translatory movement in the field of the microscope. This movement, however, is not to be compared with that of *Trypanosoma vivax* in point of rapidity or range. The rapid vibratory movement of the body and flagellum sometimes slows down for a perceptible fraction of time; but this trypanosome does not become completely quiescent as is commonly the case with *Trypanosoma pecorum*. The cell-contents are clear and homogeneous, without any appearance of a vacuole.

(b.) *Fixed and Stained.*

Length.—This trypanosome is smaller than *Trypanosoma vivax*. The average is 16·0 as against 23·7 microns. The following table gives the average length of this trypanosome in the ox, goat, and sheep. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given:—

Table 61.—Measurements of *Trypanosoma uniforme*.

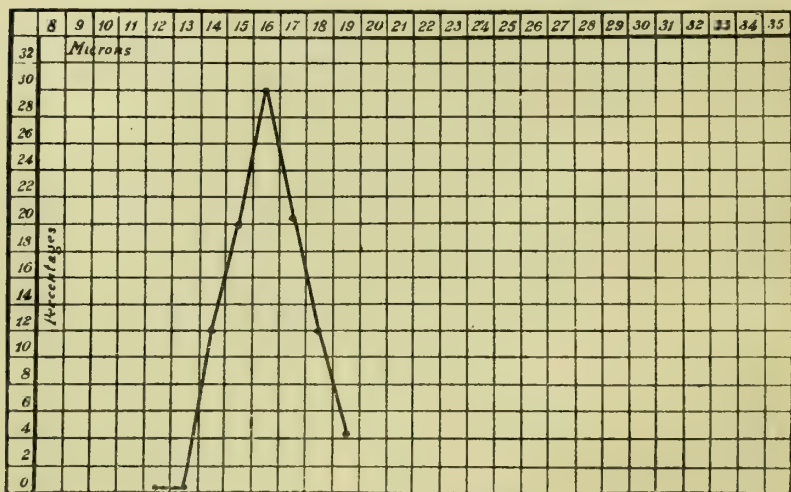
No. of experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1437	Ox	Osmic acid	Giemsa	16·0	17·5	15·0
1442	"	"	"	16·0	18·0	14·0
1442	"	"	"	16·3	19·0	14·0
1581	"	"	"	16·5	19·0	14·0
1732	"	"	"	15·5	17·5	13·5
1734	"	"	"	15·3	17·0	14·0
1689	Goat	"	"	16·4	17·5	15·0
1694	"	"	"	17·7	19·0	16·0
1694	"	"	"	14·7	16·0	13·0
1497	Sheep	"	"	17·8	19·0	16·0
1497	"	"	"	14·7	16·0	12·0
				16·0	19·0	12·0

The following table represents the distribution in respect to length of 200 individuals of this species of trypanosome:—

Table 62.—Distribution in Respect to Length of 200 Individuals of *Trypanosoma uniforme*.

No. of experiment.	Microns.								Average, in microns.
	12.	13.	14.	15.	16.	17.	18.	19.	
1437	—	—	—	5	10	5	—	—	16·0
1442	—	—	3	2	9	4	2	—	16·0
1442	—	—	1	6	5	5	1	2	16·3
1581	—	—	1	3	8	2	4	2	16·5
1734	—	—	4	8	6	2	—	—	15·3
1689	—	—	—	3	6	11	—	—	16·4
1694	—	—	—	—	2	5	9	4	17·7
1694	—	1	8	7	4	—	—	—	14·7
1497	—	—	—	—	4	7	8	1	17·8
1497	1	—	7	6	6	—	—	—	14·7
Totals ...	1	1	24	40	60	41	24	9	—
Percentages	0·5	0·5	12·0	20·0	30·0	20·5	12·0	4·5	—

Chart giving Curve representing the Distribution, by Percentages, in respect to Length of *Trypanosoma uniforme*.



Breadth.—Varies from 1·5 to 2·5 microns.

Shape.—This species of trypanosome seems to differ in shape from *Trypanosoma vivax* in that there is not the marked narrowing or constriction opposite the nucleus. The posterior extremity is rounded or blunt, and in this resembles *Trypanosoma vivax* (see Plate 9).

Contents of Cell.—Resembles *Trypanosoma vivax* in showing the appearance of clear protoplasm with fine alveolar structure.

Nucleus.—Oval in shape and compact; not, as a rule, situated in a narrowed part of the body or waist, as in *Trypanosoma vivax*. It also seems to be placed about the centre of the body, and does not take up the whole width of the cell as in the closely-allied species.

Micronucleus.—Resembles *Trypanosoma vivax* in being large, round, and terminal.

Undulating Membrane.—Narrow and little developed, as in *Trypanosoma vivax*.

Flagellum.—There is a well-marked flagellum, the free part varying from 2 to 5 microns in length.

Animals susceptible to Trypanosoma uniforme.

Table 63.

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
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CATTLE.

1909.					
Aug. 7	1442	Nat. infec.	?	5	Died of <i>T. uniforme</i> .
" 30	1581	"	?	61	Killed.
" 11	1732	"	?	79	Died of <i>T. uniforme</i> .
" 11	1734	"	?	80	Killed.

GOAT.

Sept. 1	1491	Ox 1581	15	18	No <i>post-mortem</i> .
" 16	1689	"	18	34	" "
" 18	1694	Goat 1491	9	35	" "
" 24	1716	Ox 1581	—	—	Never showed trypanosomes; under observation 40 days.
		Average	14	29	

SHEEP.

Sept. 16	1497	Ox 1581	18	46	Killed.
" 1	1601	"	—	—	Never showed trypanosomes; died 10 days after inoculation.
" 24	1717	"	—	—	Never showed trypanosomes; died 13 days after inoculation.

MONKEY.

Sept. 1	1653	Ox 1581	—	—	Experiment stopped after 34 days.
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DOG.

Sept. 1	1600	Ox 1581	—	—	Experiment stopped after 34 days.
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* Duration includes the days of incubation; it dates from the day of infection.

Table 63—continued.

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
1909, Sept. 1	1599	Ox 1581	—	—	Experiment stopped after 34 days.
Sept. 1	1597	Ox 1581	—	—	Experiment stopped after 34 days.
Sept. 1	1598	Ox 1581	—	—	Experiment stopped after 34 days.

* Duration includes the days of incubation ; it dates from the day of infection.

Cultivation of Trypanosoma uniforme.

No attempt was made to cultivate *Trypanosoma uniforme*.

Carrier of Trypanosoma uniforme.

No experiments were made in the laboratory with *Glossina palpalis* as a carrier of *Trypanosoma uniforme*, and no evidence is to hand as to what a carrier is.*

Conclusions.

1. *Trypanosoma uniforme* resembles *Trypanosoma vivax* in shape and general appearance, but differs markedly in size.
2. It also resembles *Trypanosoma vivax* in not being pathogenic to the smaller laboratory animals.
3. There is no evidence available, as in the case of *Trypanosoma vivax*, as to what the carrier of *Trypanosoma uniforme* is.

DESCRIPTION OF PLATE.

PLATE 9.

Trypanosoma uniforme in the ox, goat, and sheep. Fixed osmic acid, stained Giesma, $\times 2000$. Note the large round terminal micronucleus, the oval compact nucleus, situated about the centre of the body, the narrow undulating membrane, and the free flagellum.

30.—TRYPANOSOMA NANUM (LAVERAN).†

Introduction.

Only two cattle (Experiments 503 and 1118) examined by the Commission at Mpumu were found to harbour this trypanosome in their blood. Both came from the Uganda Company's estate at Namukekera, some fifty miles from Lake Victoria, and both had become infected on the estate.

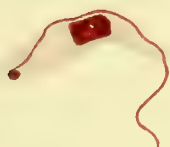
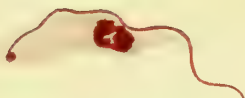
This species differed from *Trypanosoma pecorum*, in that it did not affect the smaller laboratory animals, such as monkeys, dogs,

* Since this paper was written two experiments have been carried out by the Commission (see p. 32 of this Report).

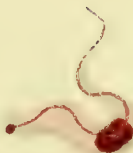
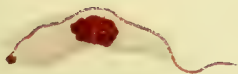
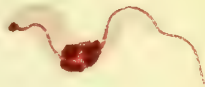
† Reprinted from the 'Proceedings' of the Royal Society, B, vol. 83, 1911.



Oxen



Goats



Sheep

rats, or mice. Guinea-pigs were also refractory to the disease. Horses, mules, donkeys, and rabbits were not available at Mpumu, so that it is impossible to say what would have been the effect of inoculation in them. Oxen and goats were inoculable, but the only sheep experimented on failed to become infected, although inoculated with the same blood which infected a goat.

It seems rash to recognise *Trypanosoma nanum* as a Uganda species on the insufficient evidence at our disposal; but yet the fact remains that this trypanosome in every case failed to infect the smaller animals, and, moreover, by placing these cases on record it will draw the attention of future workers to its possible presence.

Morphology of Trypanosoma nanum.

(a.) *Living, Unstained.*

Trypanosoma nanum is indistinguishable from *Trypanosoma pecorum* in the fresh and living condition.

(b.) *Fixed and Stained.*

Length.—The following table gives the average length of this trypanosome in the ox and goat. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given:—

Table 64.—Measurements of *Trypanosoma nanum*, Uganda, 1909.

No. of experiment.	Animal.	Method of fixing.	Method of staining.	In Microns.		
				Average length.	Maximum length.	Minimum length.
503	Ox	Osmic acid	Giemsa	13·3	15·0	11·0
983				13·1	14·0	12·0
698	Goat	"	"	13·9	16·0	13·0
698	"	"	"	15·2	16·0	12·0
882	"	"	"	12·9	15·0	11·0
883	"	"	"	13·3	15·0	11·0
1691	"	"	"	13·7	16·0	12·0
				13·6	16·0	11·0

The average length of *Trypanosoma nanum* corresponds closely with that of *Trypanosoma pecorum*, which was 13·3 microns. The question arises as to whether it would not be better for the sake of simplicity to include, for the present at least, this species in that of *Trypanosoma pecorum*. If the morphology is the same, if the important domestic animals are susceptible to both, if the geographical distribution is the same, and if the carrier is found to be the same, there seems little need of separating the two under different names.

Breadth.—At the widest part from 1·5 to 2·5 microns.

Shape.—Much the same as *Trypanosoma pecorum*, but perhaps slightly narrower, due to the less development of the undulating membrane (see Plate 11).

Contents of Cell.—As a rule homogeneous,

Nucleus.—Oval and situated about the middle of the body.

Micronucleus.—Small and rounded, and situated near the posterior extremity.

Undulating Membrane.—Perhaps a little narrower and simpler than in *Trypanosoma pecorum*. It was thought to be applied more closely to the body and less thrown into folds than in that species.

Flagellum.—There is no free flagellum.

From a comparison of Tables 65 and 66, the Chart, and the coloured figures of the two species, it is evident that *Trypanosoma pecorum* and *Trypanosoma nanum* resemble each other very closely morphologically.

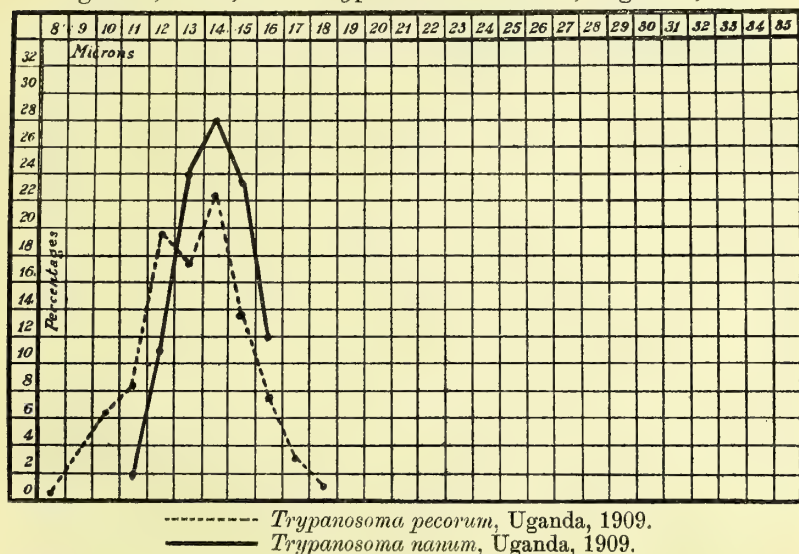
Table 65.—Showing the Distribution in respect to Length of 100 Individuals of *Trypanosoma nanum*, Uganda, 1909.

No. of experiment.	Microns.						Average, in microns.
	11.	12.	13.	14.	15.	16.	
698	—	—	7	8	4	1	13·9
698	—	1	—	2	7	10	15·2
882	1	5	10	3	1	—	12·9
883	1	4	5	7	3	—	13·3
1691	—	1	2	8	8	1	13·7
Totals ...	2	11	24	28	23	12	—
Percentages	2·0	11·0	24·0	28·0	23·0	12·0	—

Table 66.—Showing the Distribution in respect to Length of 260 Individuals of *Trypanosoma pecorum*, Uganda, 1909.

No. of experi- ment.	Microns.											Average, in microns.
	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	
82	—	—	3	4	9	3	1	—	—	—	—	11·7
505	—	—	2	2	9	4	1	2	—	—	—	12·3
—	—	—	—	2	3	6	6	3	—	—	—	13·2
593	—	—	1	—	2	1	3	7	5	1	—	14·5
44	—	—	4	2	5	3	6	—	—	—	—	12·2
559	—	—	—	2	9	4	4	—	1	—	—	12·7
461	—	—	—	—	—	5	4	1	2	6	2	15·3
543	—	—	—	2	—	2	6	6	4	—	—	14·3
1406	—	—	—	1	5	2	4	6	2	—	—	13·7
551	1	—	3	3	3	3	3	2	1	—	1	12·6
626	—	—	3	2	3	2	7	3	—	—	—	12·8
398	—	—	—	—	—	1	9	6	4	—	—	14·6
398	—	—	1	1	3	9	4	—	1	1	—	13·1
Totals ...	1	--	17	21	51	45	58	36	20	8	3	—
Percent- ages }	0·4	—	6·5	8·1	19·6	17·4	22·3	13·9	7·6	3·1	1·1	—

Chart giving Curves representing the Distribution, by Percentages, in respect to Length of *Trypanosoma pecorum*, Uganda, 1909, and *Trypanosoma nanum*, Uganda, 1909.



Comparison of Trypanosoma nanum, Uganda, 1909, with Trypanosoma nanum, Sudan, 1904.

Thanks to Dr. Andrew Balfour, the Director of the Wellcome Research Laboratories, Khartoum, the Commission is enabled to compare the Uganda strain with the original Sudan strain.

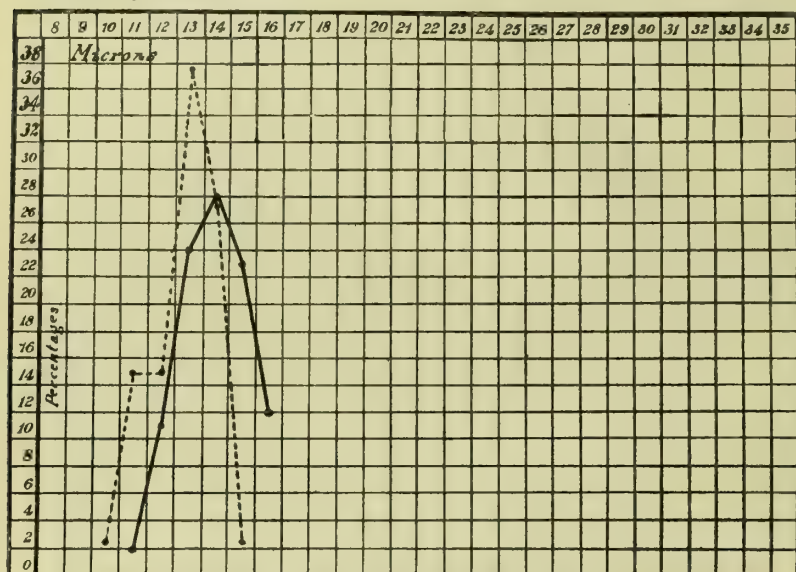
Table 67.—Measurements of Dr. Balfour's Sudan Strain of *Trypanosoma nanum*, 1904.

Animal.	Method of fixing and staining.	In Microns.		
		Average length.	Maximum length.	Minimum length.
Ox	...	12.9	15.0	11.0
"	...	12.7	14.0	10.0

Table 68.—Showing the Distribution in respect to Length of 40 Individuals of *Trypanosoma nanum*, Sudan, 1904.

Animal.	Microns.						Average, in microns.
	10.	11.	12.	13.	14.	15.	
Ox	—	3	4	6	6	1	12.9
"	1	3	2	9	5	—	12.7
Totals	1	6	6	15	11	1	—
Percentages	2.5	15.0	15.0	37.5	27.5	2.5	—

Chart giving Curves representing the Distribution, by Percentages, in respect to Length of *Trypanosoma nanum*, Uganda, 1909, and Dr. Balfour's *Trypanosoma nanum*, Sudan, 1904.



———— *Trypanosoma nanum*, Uganda, 1909.

----- *Trypanosoma nanum*, Sudan, 1904.

From a comparison of these tables and the figures in Plate 10 there can be little doubt as to the identity as regards morphology of *Trypanosoma nanum*, Uganda, 1909, and Dr. Balfour's *Trypanosoma nanum*, discovered in the Sudan in 1904:—

Animals susceptible to Trypanosoma nanum, Uganda, 1909.

Table 69.

Date	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
CATTLE.					
1909.					
Feb. 5	503	Nat infec.	?	245	From Namukekera. Died of <i>T. nanum</i> .
June 25	1118	"	?	3	From Namukekera. Died of <i>T. nanum</i> .
Sept. 29	780	Ox 983	—	—	Never showed trypanosomes; still alive after 47 days.
June 11	982	Ox 503	—	—	Never showed trypanosomes; still alive after 141 days.
" 11	983	"	20	141	Killed.
GOAT.					
Apr. 12	698	Ox 503	25	137	Died of <i>T. nanum</i> .
May 21	882	"	26	91	" "
" 21	883	"	6	46	" "
Sept. 16	1691	Oxen 503 & 982	8	12	" "
Average...			16	71	

* Duration includes the days of incubation; it dates from the day of infection.

Table 69—*continued*.

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
1909.					
SHEEP.					
May 21	880	Ox 503	—	—	Never showed trypanosomes; still alive after 82 days.
MONKEY.					
Apr. 10	687	Ox 503	—	—	Died; under observation 23 days.
June 26	1168	Ox 1118	—	—	Never showed trypanosomes; still alive after 141 days.
" 26	1169	"	—	—	Never showed trypanosomes; still alive after 97 days.
DOG.					
May 21	884	Ox 503	—	—	Died; under observation 66 days.
June 30	1194	Goat 883	—	—	Died; under observation 42 days.
" 30	1195	"	—	—	Never showed trypanosomes; still alive after 43 days.
" 30	1196	"	—	—	Died; under observation 26 days.
GUINEA-PIG.					
Apr. 10	689	Ox 503	—	—	Never showed trypanosomes; still alive after 59 days.
June 26	1162	Ox 1118	—	—	Never showed trypanosomes; still alive after 36 days.
" 26	1163	"	—	—	Never showed trypanosomes; still alive after 36 days.
" 26	1164	"	—	—	Never showed trypanosomes; still alive after 36 days.
RAT.					
Feb. 9	529	Ox 503	—	—	Experiment stopped; alive after 44 days.
" 9	530	"	—	—	Experiment stopped; alive after 44 days.
Apr. 10	688	"	—	—	Experiment stopped; alive after 59 days.
" 12	717	"	—	—	Experiment stopped; alive after 57 days.
June 26	1165	Ox 1118	—	—	Experiment stopped; alive after 57 days.
" 26	1166	"	—	—	Experiment stopped; alive after 57 days.
" 26	1167	"	—	—	Experiment stopped; alive after 57 days.
May 21	885	Goat 698	—	—	Experiment stopped; alive after 80 days.
MOUSE.					
Apr. 10	690	Ox 503	—	—	Experiment stopped; alive after 59 days.

* Duration includes the days of incubation; it dates from the day of infection.

Cultivation of Trypanosoma nanum.

No attempt was made at Mpumu to cultivate *Trypanosoma nanum*.

The Carrier of Trypanosoma nanum.

Of the two experiments made at Mpumu with *Trypanosoma nanum* and *Glossina palpalis*, one was unsatisfactory and the other negative. Since the two oxen supposed to be suffering from *Trypanosoma nanum* disease came from Namukekera, where it is stated there are no tsetse flies, it is probable that *Trypanosoma nanum*, like *Trypanosoma pecorum*, is carried by some species of biting fly other than *Glossina*.

Conclusions.

1. *Trypanosoma nanum* is indistinguishable from *Trypanosoma pecorum* either in the living condition or when fixed and stained.

2. It differs from *Trypanosoma pecorum* in not being pathogenic to the smaller laboratory animals.

3. The carrier of *Trypanosoma nanum* is probably the same as that of *Trypanosoma pecorum*, as both diseases occur under the same conditions, but there is no evidence available as to what the carrier is.

DESCRIPTION OF PLATES.

PLATE 10.

Figures in black and white for the comparison of *Trypanosoma nanum*, Uganda, 1909, and *Trypanosoma nanum*, Sudan, 1904. $\times 2000$.

PLATE 11.

Trypanosoma nanum, Uganda, 1909, in the goat. Fixed osmic acid, stained Giesma. $\times 2000$.

31.—TRYPANOSOMA INGENS, SP. NOV.

This species of cattle trypanosome was described and figured in the 'Proceedings' of the Royal Society, B, vol. 81, 1909, p. 323, and reprinted in the Sleeping Sickness Commission Reports, No. X., p. 27.

32.—TRYPANOSOMA GALLINARUM, SP. NOV.

Introduction.

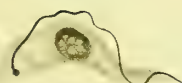
In November, 1909, Mathis and Leger noted the occurrence of a trypanosome in the blood of a domestic fowl in Tonkin. It is described as being relatively short and stumpy—36 microns long and 4.5 microns in breadth.

In January, 1910, the Sleeping Sickness Commission were working with domestic fowls in Uganda, to ascertain if they could be infected with *Trypanosoma gambiense*. In the course of these investigations a large trypanosome, over 60 microns in length, was observed in the centrifuged blood of a fowl. The parasite was also found in two other fowls, but only after prolonged centrifuging of their blood.

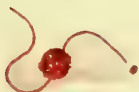
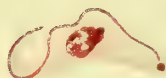
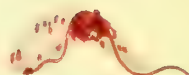
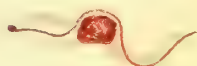
The Commission believe that this trypanosome is not of the same species as that observed in the blood of fowls in Tonkin,



T. Nannum, Uganda, 1909.









and, therefore, they propose that it should be named *Trypanosoma gallinarum*.

In order to find *Trypanosoma gallinarum* it is necessary to centrifuge about 10 c.c of fowl's blood: the "buffy" layer of white blood corpuscles lying upon the top of the deposit should be removed with a fine pipette and examined, unstained, under a cover-glass. If trypanosomes are found, films may be fixed and stained as described in the 'Proceedings'* of the Royal Society.

Morphology of Trypanosoma gallinarum.

(a.) *Living, Unstained.*

In fresh blood which has been washed and centrifuged, *Trypanosoma gallinarum* is generally observed to remain in one place, lashing its flagellum about, and performing slow sinuous movements of its body and undulating membrane.

As this parasite has never been observed, except in blood subjected to prolonged centrifugalisation, the nature of its activities under natural conditions is unknown.

(b.) *Fixed and Stained (Plate 12).*

Length.—The following table gives the length of 20 individual trypanosomes:—

Table 70.—*Trypanosoma gallinarum.*

Posterior extremity to micronucleus.	Micro-nucleus to nucleus.	Length of nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
17	9	3	27	9	65
14	10	4	30	5	63
12	12	4.5	24.5	6	59
14	12	3.5	24.5	9	63
12	11	5	27	8	63
11	12	3.5	21.5	4	52
10	12	3.5	23.5	7	56
11	13	2.5	28.5	10	65
13	14	3	25	9	64
9	11	3	32	8	63
11	14	3	24	8	60
9	14	2.5	26.5	9	61
13	13	4	24	11	65
13	10.5	3.5	24	7	58
11	12	4	24	9	60
11	12	4	27	10	64
10	15	4.5	27.5	8	65
10	12	5	24	9	60
13	11	3	25	11	63
10	11	3	21	13	58
Average 11.7	12.0	3.6	25.5	8.5	61.3

It is shown in the above table that this trypanosome varies in length between 65 and 52 microns. The majority of them are over 60 microns long. Short or stumpy forms were never seen.

* B, vol. 81, 1909, pp. 16 and 17.

Breadth.—As measured across the broadest part of the body, the breadth varies between 5 and 7 microns.

Shape.—The body of this species has the spindle shape, typical of the genus *Trypanosoma*. The posterior or anti-flagellar extremity is pointed and is prolonged far beyond the micronucleus. The anterior or flagellated extremity tapers gradually to end in the free flagellum.

Contents of cell.—The body substance is reticular and is ribbed longitudinally by parallel lines, described as myonemes, which extend from the flagellar extremity to the micronucleus. Here and there a few unstained spots, resembling vacuoles, may be seen.

Nucleus.—Band-like in form, situated near the middle of the body of the trypanosome; and running across its long axis the nucleus, stained with Giemsa, shows faintly pink, and appears to be homogeneous in structure.

Micronucleus.—Situated 10 to 17 microns from the posterior (anti-flagellar) end, oblong in shape, from 1 to 1.5 microns in the long diameter, and staining a deep red with Giemsa.

Undulating membrane.—Is broad and thrown into many folds.

Flagellum.—Takes origin near the micronucleus, and after following all the sinuities in the margin of the undulating membrane, ends in a free lash 6 to 10 microns long.

Animals susceptible to Trypanosoma gallinarum.

Experiment 2052.

January 10, 1910. A few drops of centrifuged blood from a fowl, Experiment 1926, which was infected with *Trypanosoma gallinarum*, were inoculated into another fowl, Experiment 2052.

February 16. Fowl, Experiment 2052, was killed: its blood was centrifuged, and *Trypanosoma gallinarum* was found in it.

The necessity of killing the fowls and centrifuging their blood before this parasite can be found, precludes the possibility of doing satisfactory sub-inoculation experiments with birds that have been running about for some time. Newly-hatched chickens were not available when these experiments were in progress.

The blood of two fowls (Experiments 1926 and 1927) was injected into monkeys, with negative results.

Cultivation of Trypanosoma gallinarum.

This parasite grows readily on Novy and MacNeal's blood-agar medium made up with fowl's blood. The following experiment is cited as an example.

Experiment 2198.

February 16, 1910. Inoculated six tubes with a drop or two of defibrinated fowl's blood. The trypanosomes were very scarce in the blood.

February 21. Tube 1 shows a fair number of active flagellates, resembling *Crithidia* (Plate 13, figs. 2 and 4).

February 23. Tube 1, very active flagellates, with long and very fine free flagella are seen (Plate 13, figs. 1, 2, 3, 4, 5, and 6). They move rapidly across the field, flagellar end foremost. When meeting with an obstruction they will at once move off in the opposite direction, anti-flagellar end foremost. In the fresh preparation no undulating membrane can be made out. Many of the trypanosomes are oat-shaped, vacuolated, and motionless.

February 26. The following forms of trypanosomes are noted : (1) Torpedo-shaped bodies, dashing about the field flagellar end foremost; (2) many forms similar to the above, but showing longitudinal division of the trypanosomes, commencing at the flagellar end (Plate 13, figs. 10 and 11); (3) oval and circular forms, with single or double macro- and micronucleus, and with one or more active flagella (Plate 13, figs. 7 and 8).

March 3. Fifteen days after the tube was inoculated. Many oval parasites are seen grouped together in the form of rosettes, with flagella directed to the centre (Plate 13, fig. 9). Large non-motile masses of protoplasm, of irregular shape and much vacuolated, are also seen.

The trypanosomes remained alive and multiplied up to the 25th day, when the tube dried up and was thrown away. In another experiment (2288) the trypanosomes remained alive in the culture tube for 36 days.

(a.)—*Living, Unstained.*

Size.—The cultural forms of *Trypanosoma gallinarum* vary in size. The rapidly moving forms, which in the fresh preparation resemble minute torpedoes or tadpoles, are from 25 to 35 microns long. Oval and circular forms vary from 5 to 15 microns in diameter.

Shape.—As noted above.

Contents of Cell.—In the living unstained condition many coarse granules and large vacuoles are seen within the cell.

Undulating Membrane.—In the elongated forms furnished with a flagellum, a narrow undulating membrane can generally be made out.

Flagellum.—A very long rapidly-vibrating free flagellum is usually present.

(b.)—*Fixed and Stained* (Plate 13).

Protoplasm.—Is granular, and contains vacuoles and many particles of material, staining a bright-red colour with Giemsa's stain.

Nucleus.—Stains bright red. It is usually seen either as a compact homogeneous mass of chromatin, or in the form of an irregular-shaped clump of coarse granules.

Micronucleus.—Is difficult to distinguish. It stains a deep red, and is usually situated alongside the nucleus, or anterior to it, *i.e.*, on its flagellar side.

Flagellum.—Is a very long and fine filament, difficult to stain.

The Carrier of Trypanosoma gallinarum.

The carrier of *Trypanosoma gallinarum* is unknown. Lice or the tick, *Argus persicus*, may be suggested. Both are common parasites of fowls in Uganda. This trypanosome did not multiply or develop in the intestines of *Glossina palpalis* fed on the infected fowls.

Conclusion.

Trypanosoma gallinarum is a well-defined species of avian trypanosome and, so far as is at present known, it is non-pathogenic to fowls.

DESCRIPTION OF PLATES.

PLATE 12.

Five typical specimens of *Trypanosoma gallinarum* are here represented, as seen in the centrifuged flood of a fowl. The specimens were fixed in osmic acid, stained by Giemsa's stain, and magnified 2,000 diameters. Note the tapering posterior (anti-flagellar) extremity projecting far beyond the micronucleus, the longitudinal striations of the body, the pale-stained macronucleus, and the unstained spots which are believed to be deep-seated vacuoles.

PLATE 13.

Figs. 1 to 6, represent motile forms of *Trypanosoma gallinarum* as seen in artificial cultures of 5 to 7 days' growth. Stained Giemsa, $\times 2000$.

Figs. 7 and 8, represent oval and circular forms, 10 days' growth.

Fig. 9, aggregation or "rosette" form of *Trypanosoma gallinarum* after 15 days' growth. The flagella are all directed to the centre of the "rosette" in this specimen. Sometimes the flagella are arranged around the periphery of the "rosette."

Figs. 10 and 11, represent dividing forms of *Trypanosoma gallinarum*.







E. EXPERIMENTS DESIGNED TO DISCOVER THE INSECT CARRIERS OF THE TRYPANOSOME DISEASES OF DOMESTIC ANIMALS IN UGANDA.

33.—GLOSSINA PALPALIS AS A CARRIER OF TRYPANOSOMA VIVAX IN UGANDA.

This paper was published in the 'Proceedings' of the Royal Society, B, vol. 82, 1909, p. 63, and reprinted in the Reports of the Sleeping Sickness Commission, No. X., p. 63.

The conclusions come to were that *Glossina palpalis* on the shores of Lake Victoria are infected, not only with *Trypanosoma gambiense* but also by *Trypanosoma vivax*. What the reservoir of the virus of *Trypanosoma vivax* is, is not fully known, but that the buffalo, water-buck, and other antelope which live on the Lake-shore are suspected. This trypanosome has been found on one occasion in a bush-buck.

34.—EXPERIMENTS TO ASCERTAIN IF CERTAIN TABANIDÆ ACT AS THE CARRIERS OF TRYPANOSOMA PECORUM.*

Introduction.

The study of the trypanosome diseases of domestic animals in Uganda was a branch, and an important one, of the work undertaken by the Commission. Among other points arising in this investigation, the finding, if possible, of a carrier of the various trypanosome diseases was necessary. In the 'Proceedings'† of the Royal Society, the Commission published a paper entitled "Trypanosome Diseases of Domestic Animals in Uganda. I.—*Trypanosoma Pecorum*." This paper gives full details as to the definition, morphological and cultural characteristics, and distribution in Africa, of *Trypanosoma pecorum*. A few experiments and experiences of the Commission were also given, which led to the belief that possibly *Tabanidæ* were the common carriers in Africa of the disease caused in cattle by this trypanosome.

With the object of gaining further knowledge on this important problem, the Commission worked in Uganda from January to July, 1910, on the following lines:—

1. An investigation of the biting flies occurring in the vicinity of the laboratory at Mpumu, Chagwe, Uganda.
2. A study of the natural history of these biting flies.
3. Transmission experiments with these flies.
4. A study of the flagellates, if any, natural to these flies.

* Reprinted from the 'Proceedings' of the Royal Society, B, vol. 83, 1911.

† B, 1910, vol. 82, p. 468; also p. 128 of this Report.

1. *An Investigation of the Biting Flies Occurring in the Vicinity of the Laboratory at Mpumu, Chagwe, Uganda.*

No systematic search was made for blood-sucking flies in this neighbourhood till January, 1910. Up to this date the Commission had identified some four different species in the vicinity of Mpumu.

An outbreak of trypanosome disease in cattle having occurred in September, 1909,* in the valleys surrounding Mpumu hill, it was decided to make a prolonged and careful search for biting flies in these valleys. Boys were employed whose sole duty it was to hunt daily for these flies, cattle accompanying the boys to act as a bait for the flies. This more systematic search produced unexpected results.

In the place of only three or four varieties of biting flies, no less than 12 species of *Tabanidæ*, five species of *Hæmatopota*, two different *Chrysops*, a new and very handsome *Rhinomyza*, and *Glossina palpalis*, were captured within a 3-mile radius of the laboratory. These flies were kindly identified for us by Mr. E. E. Austen, F.L.S., to whom specimens were sent, and for whose help and courtesy the Commission is much indebted. The full list is here given, as it will probably be of value to future workers in the Mpumu laboratory, and of interest to entomologists.

Tabanidæ.

- Tabanus secedens* (Walk.), very numerous.
- Tabanus fuscomarginatus* (Ricardo), very numerous.
- Tabanus thoracinus* (Pal. de Beauv.), very numerous.
- Tabanus par* (Walk.), fairly numerous.
- Tabanus tæniola* (Pal. de Beauv.), fairly numerous.
- Tabanus socialis* (Walk.), fairly numerous.
- Tabanus variatus* (Walk.), fairly numerous.
- Tabanus fasciatus* (Fabr.), sub. sp. *niloticus* (Austen).
- Tabanus insignis* (Lw.), scarce.
- Tabanus variabilis* (Lw.), scarce.
- Tabanus obscurissimus* (?), scarce.
- Tabanus irroratus* (Surcouf), rare.

Hæmatopota.

- Hæmatopota ugandæ* (Ricardo). *H. vittata* (Lw.), syn. *pulchrior* (Austen). *H. fusca* (Austen). *H. brunnescens* (Ricardo). *H. similis* (Ricardo).
- (All numerous.)

Chrysops.

- Chrysops funebris* (Austen). *C. silacea* (Austen).

Glossina.

- Glossina palpalis* (Rob.-Desv.).

* See 'Proceedings' of the Royal Society, B, 1910, vol. 82, p. 477; also p. 137 of this Report.

*Rhinomyza.**Rhinomyza perpulcra* (Austen).

This part of the investigation made two facts clear to the Commission. Firstly, that experiments to ascertain a carrier of any of the trypanosome diseases of domestic animals in Africa must necessarily be both prolonged and difficult. Secondly, that the distribution of the different species of biting flies in any country cannot be mapped out by a travelling entomologist. Accurate maps can only be made by the whole-hearted co-operation of those officials and settlers who are resident in the country for long periods. The observation should, where possible, be made daily, and continued for at least one year in the same locality.

2. *A Study of the Natural History of the Biting Flies Found in the Neighbourhood of Mpumu.*

It should be clearly understood that whatever observations are given below refer to the natural history of these flies as observed in this locality only. Elsewhere difference in climatic and other conditions may give rise to variations in the habits and life-history of these flies.

Glossina palpalis.

The habits of the *Glossina* in this neighbourhood do not vary from the same species on the Lake-shore. Their presence was, however, both interesting and disquieting, as the Kasala stream, where they were captured, is at least 6 miles from the nearest point of Lake Victoria. Further, the stream has no connection whatever with this Lake. Rising in a narrow belt of forest at the foot of Mpumu hill, it flows through forest along the north-west face of the hill and then joins the Sezibwa River, which trends northwards, running parallel with the Nile, to empty into Lake Chioga. The *Glossina* were very scarce, three or four only being caught monthly between January and July, 1910. They were all caught at one spot—a ford of the stream, situated a mile or so from the laboratory. No tsetse-flies were seen in any other part of the stream. It is possible these flies had found their way down from the hill, since it was by no means an uncommon circumstance for some flies to escape from their cages when brought up by the fly-boys. Escaped flies were found on several occasions on different parts of the hill.

The Tabanidæ.

Habitat.—Narrow belts of dense tropical forest, which commonly mark the windings of a flowing stream. The two essential factors appear to be deep shade and water, not necessarily running water. These flies are not found on grass-covered hill-tops.

Habits.—Emerging from the upper branches of the forest trees, on which they presumably shelter during the night, these flies

commence feeding about 7 a.m. if the morning be fine with plenty of sunshine. They cease to feed about 6 p.m. Their most active hours are from 11 a.m. to 1 p.m., viz., the hottest part of the day. From 6 a.m. to 8 a.m. and again from 4 p.m. to 6 p.m., they are sluggish and do not feed readily. In wet, cold, or dull weather no flies are to be seen as a rule. They bite cattle and man freely, but much prefer the former, and will rarely attack man in the presence of cattle. Their bite is like the stab of a sharp lancet, and draws a free trickle of blood from both oxen and man. They have followed cattle a distance of 3 miles to the laboratory, but invariably left the hill-top soon after their arrival, or died in sheltered spots about the houses a few days after their arrival.

Variation in Incidence.—This phenomenon is the most striking characteristic of the *Tabanidæ*. A forested stream known to be free from a certain species of *Tabanus* one day will be infested with that species during the next two or three days. This eruption, if it may be so called, appears to be simultaneous over a given area, and may be explained by the flies all arriving at maturity about the same day. This tends to show that the females all lay their batches of eggs about the same time. Further, it is not unreasonable to date the commencement of the fly's winged life from this time, and so, watching carefully for their final disappearance, we arrive, probably with some degree of accuracy, at the length of life of the fully-developed fly. The disappearance of the fly is almost as sudden as their eruption; in some three days a locality previously swarming with a certain species of *Tabanus* will be quite free from this species. In this manner the Commission have observed carefully three species of *Tabanidæ*, viz., *Tabanus secedens*, *Tabanus fuscomarginatus*, and *Tabanus thoracinus*. In these cases on the above computation, the limit of life of these flies is as follows:—*Tabanus secedens*, four months; *Tabanus fuscomarginatus*, two months; *Tabanus thoracinus*, four months. At least two such eruptions of the same fly may occur in the same locality in the same year.

Sex.—During the seven months (January-July, 1910) these experiments were taking place, many thousand *Tabanidæ* were captured, but no male of any of the 12 different species was ever seen or taken.

The Hæmatopota.

Habitat.—The *Hæmatopota* have a more extensive range than the *Tabanidæ*. They are to be found in the same localities as the *Tabanidæ*, and also in large numbers, in open swampy areas where few or no trees grow. The rank grass which grows waist-high in these swamps, appears to satisfy what need they may have for shade. Another very favourite locality for these flies is a road or ford crossing streams and swamps. The fly is absent from dry, open, grass-covered hill tops.

Habits.—The hours of greatest activity, and the feeding times of this genus, correspond to those given above for the *Tabanidæ*. They have a sluggish flight, and attack man more readily than do the *Tabanidæ*. When they settle on their victim they rarely bite

at once, but usually walk about slowly for a few seconds before inserting their proboscis. They will follow cattle or man for a mile or two.

Sex.—No male fly was ever captured by the Commission during the time these observations were taking place.

The Chrysops and the Rhinomyza.

These flies were comparatively scanty in the neighbourhood, and no accurate observations were made of their natural history. All were caught at the ford on the Kasala stream, at the foot of Mpumu hill. *Rhinomyza perpulcra* appeared to be very localised in its distribution, as it was never seen elsewhere in the vicinity. It was usually captured whilst sunning itself on stones in the stream, or whilst it was biting natives washing clothes at this ford. Its habitat appeared to be the densely forested banks of the ford. Mr. Austen informs us that it is a new African species.

3. *Experiments to Ascertain if Tabanus secedens (Walk.), Tabanus fuscomarginatus (Ricardo), or Tabanus thoracinus (Pal. de Beauv.), are Capable of Transmitting Trypanosoma pecorum from Sick to Healthy Oxen.*

The experiments were carried out between March and August, 1910. The above-named species were used, as they were the most common among the *Tabanidæ* during this period. Only wild flies were employed, as the Commission never succeeded in finding the eggs or larvæ of these flies. The species of *Tabanus* used in any experiment, and the duration of the experiment, were thus entirely dependent on the seasonal incidence of the fly in the neighbourhood.

Endeavours were at first made to carry out the experiments in the laboratory on Mpumu hill. Cages, some 12 inches square, were made, with sides of wood, the top and bottom consisting of wide-meshed mosquito netting. The captured flies were placed in these cages—about 50 flies per cage—the cages being kept over water in the laboratory when the flies were not being fed. The daily temperature of the laboratory during the days this method was tried was very constant, being 70·2° F. (21·2° C.) in the morning at 8, and 80·2° F. (26·8° C.) in the afternoon at 4. The flies were placed on oxen and monkeys for 20 minutes once daily, to give them ample opportunity to feed.

This method was not successful, the flies—*Tabanus secedens* (Walk.)—all dying within four days of being caged, the majority within 48 hours. When placed on an ox, within 12 hours of capture, a few flies fed greedily. After 24 hours in the cages no fly was observed to bite. Monkeys were never bitten by the flies, though they would feed on man as readily as on the ox when freshly caught. If shaded from direct sunlight, the flies refused to feed.

It was decided to build a fly-proof kraal in the natural haunts of the fly, in the hope that the *Tabanidæ* would live longer and feed more readily. The Kasala stream at the foot of Mpumu hill was chosen as the site of the kraal. The kraal was divided into two compartments, which were made fly-proof from each other

and from the outside. In one compartment no flies of any species had access. Into the other compartment the species of *Tabanus* under experiment were liberated—50 flies per day, on an average. In the kraal four calves were kept: three normal and one calf infected with *Trypanosoma pecorum*. The infected calf and two of the normal calves were taken from the fly-free compartment into the compartment containing the *Tabanidæ* for three hours daily. The hours chosen were 11 a.m. to 1 p.m., and 3 p.m. to 4 p.m., when the sun was hot and shining on the kraal. Every opportunity were thus given to the flies to feed on both the infected and the healthy calves. The other normal calf was utilised as a control, being kept continuously in the fly-free compartment and so protected absolutely from the bites of blood-sucking flies.

No effort was made to rid any of the calves of ticks, body lice, etc., therefore, if the normal calves which daily entered the fly-compartment with the infected calf contracted *Trypanosoma pecorum* infection whilst the control calf remained healthy, then the *Tabanus* under experiment was in all probability the transmitting agent. If the control calf also contracted the disease, then some agency other than *Tabanidæ*, such as ticks, etc., would probably be responsible. Lastly, if all the normal calves escaped infection, then presumably the *Tabanus* under experiment did not transmit the disease.

The *Tabanidæ* did not appear to live more than three or four days, in spite of the presence of running water, shrubs and foliage in the fly-compartment. The majority died within the first 48 hours. A few fed greedily on the calves for 12 to 18 hours after capture.

The normal calves and the control calf were examined bi-weekly for trypanosomes throughout the experiment, and for one month after its completion. The infected calf was also examined bi-weekly: *Trypanosoma pecorum* were always seen in its blood; sometimes they were present in large numbers, sometimes scanty.

Table 71 gives the result of these experiments at a glance:—

Table 71.

Species of <i>Tabanus</i> used.	Experiment No. of calves.	No. of days <i>Tabanidæ</i> fed on calves.	Approximate No. of flies used.	Result.
<i>Tabanus secedens</i> (Walk.).	2304 & 2305	30	1500	Negative; calves remained healthy.
<i>Tabanus fuscomarginatus</i> (Ricardo).	2400 & 2401	43	2150	" "
<i>Tabanus thoracinus</i> (Pal. de Beauv.).	2542	55	2650	" "

Remarks.—It is seen that the *Tabanidæ* had ample opportunity to feed indiscriminately on the infected and healthy calves over a considerable period of time. Large numbers of flies were used,

yet no normal calf contracted the disease. The fact that the *Tabanidæ* died within 48 hours of being put in the kraal does away with the possibility of a development of *Trypanosoma pecorum* in the flies. These experiments, therefore, only prove that these flies do not transmit the trypanosome mechanically.

4. *A Study of the Flagellates found in Tabanus secedens, Tabanus fuscomarginatus and Tabanus thoracinus.*

In this investigation two series of dissections were made. In the first wild flies caught in the neighbourhood of the laboratory were dissected. In the second series dissections were made of wild flies caught in the neighbourhood and introduced into the fly-compartment of the kraal, where they were used in the transmission experiments detailed above.

As wild flies were used throughout, it was necessary to carefully study the flagellates which may infect these flies, in order that parasites natural to the flies, such as *Crithidia* or *Herpetomonas*, should not be mistaken for developmental forms of pathogenic trypanosomes in the fly.

It should be noted here that the cattle which grazed in the neighbourhood where the flies were caught were healthy during the period that the investigations were being carried out.

In the first series of dissections 138 *Tabanus secedens* and 49 *Tabanus thoracinus* were dissected. Of the *Tabanus secedens* 7 (5 per cent.) showed a heavy infection of flagellates in the hind-gut, rectum, and proctodæum. The mouth parts and remainder of the alimentary tract contained no flagellates. Of the *Tabanus thoracinus* dissected, flagellates were found in the hind-gut, rectum and proctodæum of 13 (26·5 per cent.). The mouth parts and remainder of the gut were negative for flagellates. No *Tabanus fuscomarginatus* were dissected in this series.

The flagellates found in the hindermost portions of the intestines of both these species were similar. Two main types predominated.

Type 1, Oval, Flagellated Parasites.

(a.) *Living, Unstained.*

This type is very active, and darts rapidly across the field when free. More commonly large masses of these flagellates are seen to be irregularly grouped together, the flagella of the outermost ones rapidly vibrating. This type divides by longitudinal fission, and forms undergoing this process are commonly seen. Bright points can often be made out in the body-substance of these parasites (chromatin granules); no definite vacuoles could be seen. Masses of these flagellates sometimes occupied a whole field.

(b.) *Fixed and Stained.*

These flagellates contain a distinct macro- and micronucleus. The macronucleus is large, roughly circular and sometimes diffused. It lies about the centre of the body of the parasite, often occupying its whole width. The micronucleus is circular or rod-shaped, is placed on the flagellar side of and close to the macronucleus. The flagellum arises from the micronucleus, it is

short and projects from the body of the parasite, the protoplasm of the body being often prolonged for a short distance along the flagellum. There is no undulating membrane. Chromatin granules, irregular in number and size, are often to be seen in the vicinity of the macronucleus (Plate 14, figs. 1 to 6).

Type 2, Circular, Non-Flagellated Parasites.

These parasites contained macro- and micronuclei. The drawing clearly indicates their structure (Plate 14, figs. 7 to 14). In the fresh preparations they were motionless.

In the second series of dissections of the *Tabanidæ* which had been liberated in the fly-compartment of the kraal, 50 *Tabanus secedens*, 24 *Tabanus thoracinus*, and 37 *Tabanus fuscomarginatus* were dissected. Of the 50 *Tabanus secedens*, 2 were infected with flagellates (4 per cent.). Of the 24 *Tabanus thoracinus*, 1 was infected (4 per cent.). No *Tabanus fuscomarginatus* contained parasites. The three infected flies all contained flagellates in the hind-gut, rectum, and proctodæum, which resembled Types 1 and 2 mentioned above.

Type 3, Elongated, Flagellated Parasites.

One fly, a *Tabanus secedens*, contained many flagellates, of a different variety, in the fore-gut, mid-gut, and fore-part of hind-gut. In the fresh preparation this third type of flagellate was motile, and possessed a poorly-developed undulating membrane, which was thrown into short folds. The movements of the parasite were active, but not transitory to any great extent. The accompanying drawing (Plate 14, figs. 15 to 24) so clearly show the structure of this type in the stained state that further description is unnecessary.

Type 3 occurs principally in the thick-walled mid-gut of the fly and, to a lesser extent, in the fore-gut and fore-part of the hind-gut. Types 1 and 2 occur only in the hind-gut, rectum, and proctodæum of the fly.

Inoculation Experiments carried out with these Parasites.

Experiment 2404.

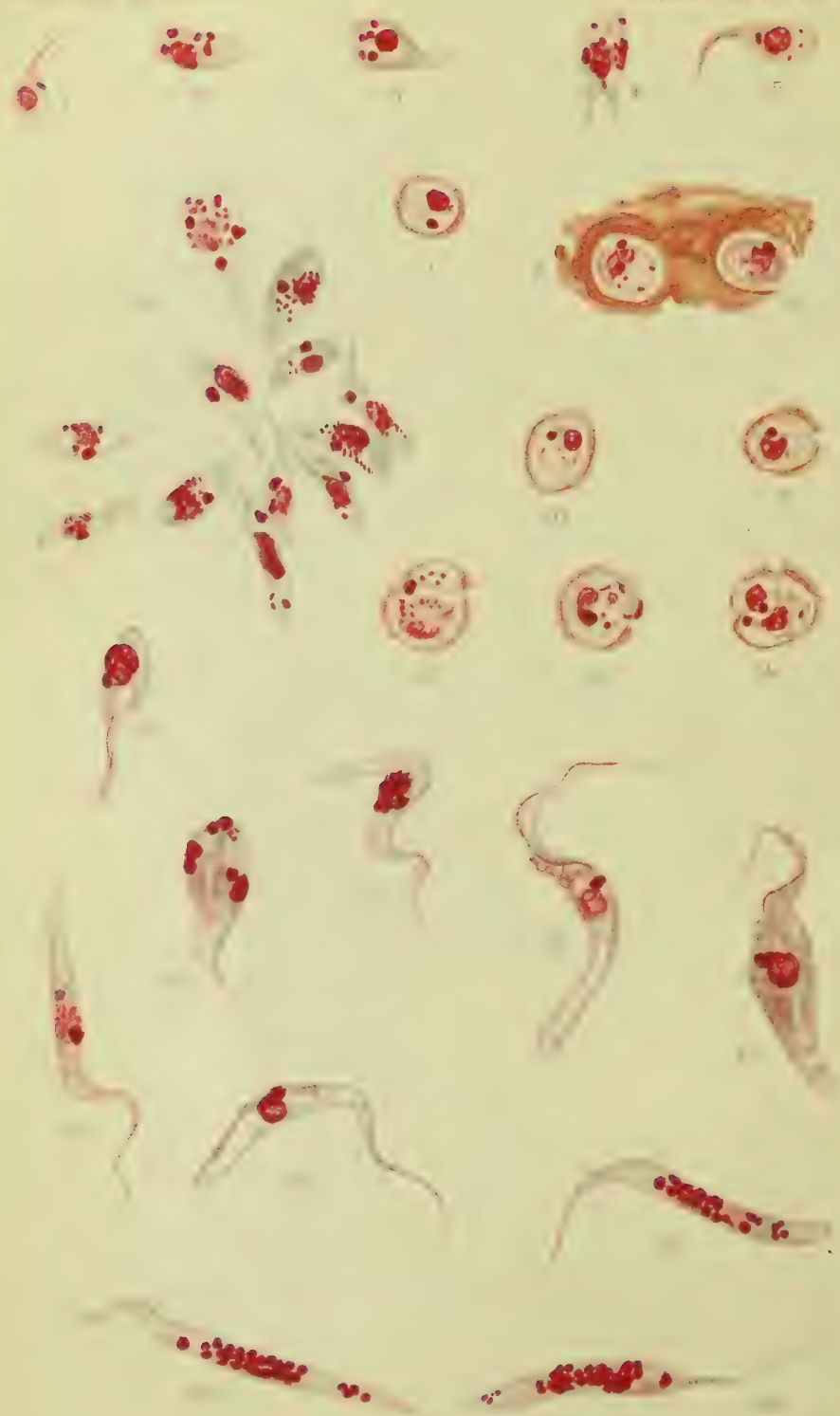
April 23, 1910.—White rat inoculated, intra-peritoneally, with contents of hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had been in fly-compartment of kraal; it was heavily infected with Type 1 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Experiment 2405.

April 23, 1910.—White rat inoculated, intra-peritoneally, with contents of fore-gut and mid-gut of a wild *Tabanus secedens*. This fly had been in kraal, and its fore-gut and mid-gut were swarming with Type 3 of the parasite in an active state.

Result.—Negative. Rat examined for one month after the injection.



Experiment 2411.

April 27, 1910.—White rat inoculated, intra-peritoneally, with hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had never been in kraal. It was heavily infected with Type 2 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Experiment 2412.

April 27, 1910.—White rat inoculated, intra-peritoneally, with hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had never been in kraal; it was heavily infected with Type 1 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Experiment 2412B.

July 4, 1910.—White rat inoculated, intra-peritoneally, with the pooled citrated contents of the hind-gut, rectum, and proctodæum of two wild *Tabanus thoracinus*. These flies had never been in kraal and were heavily infected with Types 1 and 2 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Conclusions.

1. *Tabanus secedens*, *Tabanus thoracinus*, and *Tabanus fusco-marginatus* appeared to be unable to transmit *Trypanosoma pecorum* from infected to healthy cattle by the mechanical method of transmission.

2. Owing to the short life of these tabanids in captivity it is impossible, from the above experiments, to state whether they can convey the disease (*Trypanosoma pocorum*) after a period of development of the trypanosome in the fly.

3. We believe the three types of flagellates found in *Tabanus secedens* and *Tabanus thoracinus* to be various stages in the development of a harmless Crithidium in these flies.

DESCRIPTION OF PLATE 14.

Smear preparation of hind-gut, rectum and proctodæum of a *Tabanus secedens*, stained Giemsa, $\times 2000$.

Figs. 1-6, Type 1, oval, flagellated parasites. Fig. 4 is a dividing form of this type. Fig. 1 shows the most common form of this type. Fig. 6 represents an irregular mass of the parasites; these masses often contain very large numbers of the parasites. The flagella are poorly stained in these preparations, and so do not show up well (Expt. 2412).

Figs. 7-14, Type 2, circular, non-flagellated parasites. Made from smear preparations of the rectum and proctodæum of a *Tabanus secedens* (Expt. 2411).

Figs. 15-24, Type 3, elongated, flagellated parasites. Figs. 17 and 18 show the most typical forms of this type. Fig. 19 is a stout atypical form, which was not common. Fig. 15 represents a form which would appear to be reverting to Type 1. Fig. 16 is a dividing form. The parasites figured in Figs. 15-24 were obtained from a smear made of the mid-gut of a wild *Tabanus secedens* which had been in the fly-compartment of the cattle kraal (Expt. 2405).

F. TRYPANOSOMES AND OTHER PARASITES OF ANIMALS IN UGANDA.

35.—A NOTE ON THE OCCURRENCE OF A TRYPANOSOME IN THE AFRICAN ELEPHANT (*Trypanosoma elephantis*, sp. nov.).

This paper was published in the 'Proceedings' of the Royal Society, B, vol. 81, 1909, p. 414, and reprinted in the Reports of the Sleeping Sickness Commission, No. X., p. 54.

36.—A TRYPANOSOME FOUND IN THE BLOOD OF A CROCODILE.

The occurrence of trypanosomes in the blood of crocodiles inhabiting Lake Victoria was first mentioned by Koch.*

Minchin, Gray, and Tulloch, in 1905, while searching for a vertebrate host of the trypanosome of Sleeping Sickness in Uganda, found a trypanosome in crocodile's blood, but did not describe it.

The trypanosome of crocodiles has excited some interest owing to its possible connection with *Trypanosoma grayi* found in *Glossina palpalis* (Kleine).†

It is, therefore, figured in this Report, as the Commission is not aware that a drawing of this parasite has appeared in an English publication.

The parasite was never observed in the living state.

DESCRIPTION OF PLATE 15 (CROCODILE TRYPANOSOME).

Trypanosome of crocodile, fixed osmic acid, stained Giemsa, × 2000.

Total length, 87 microns.

Posterior extremity to micronucleus, 18 microns.

Micronucleus to anterior extremity, 46 microns.

Free flagellum, 23 microns.

Note the oval nucleus stained pink and situated across the body of the parasite; immediately behind it is the micronucleus, squarely oval in outline and stained a deep red. The flagellum arises close to the micronucleus, follows the edge of a broad undulating membrane, and terminates in a long free lash. Around the nucleus there is an unstained area across which the myonemes can be seen running. Posterior to the micronucleus a few red-staining granules are to be seen, but what their nature is, is unknown. Anterior to the nucleus the body of the trypanosome is marked by longitudinal striations—the so-called myonemes—staining a deeper blue than the rest of the body.

37.—THE OCCURRENCE OF A SPIROCHÆTE IN THE BLOOD OF AN AFRICAN ANTELOPE.

The Spirochætes have never been reported as having been observed in the blood of the African game animals. It may be noted, however, that Theiler has described a species of this parasite, which is transmitted by the tick (*Boophilus decoloratus*), the common blue tick, and is the cause of disease amongst cattle in

* Report of the German Sleeping Sickness Commission, 1909.

† *Deutsche Medizinische Wochenschrift*, July 28, 1910, No. 30, pp. 1400-1403.

1.

2.

3.

4.

5.

Spirochætes.

*Trypanosome
from
Crocodile.*

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

11.

12.

M.E. Bruce, del.

Piroplasma in White Mouse.

Hutchinson stamp

the Transvaal. The Sleeping Sickness Commission occasionally observed Spirochætes in the blood of cattle in Uganda.

On April 13, 1910, a bush-buck (*Tragelaphus sylvatica*), caught in the Gomba District of Uganda (lat. $0^{\circ} 20'$, long. $32^{\circ} 10'$), was brought alive to the laboratory at Mpumu. The animal was emaciated, and was suffering from persistent diarrhœa. Its coat was rough and staring, and the creature was so weak that it could not stand. It was thought that the buck might be suffering from some trypanosome disease. Its blood was, therefore, carefully examined. No trypanosomes were found, but in the fresh unstained film a few minute silvery filaments were seen wriggling amongst the blood corpuscles, or passing rapidly across the field. In preparations of the blood, stained by Giemsa's method, the organism appeared to be a Spirochæte.

The figures on the accompanying plate (15) show the appearance presented by the stained parasite, as observed in the blood of the bush-buck. It is seen to be a comparatively thick ribbon-shaped form of Spirochæte, measuring 9 to 15 microns in length, and containing four or five spirals.

A few Spirochætes were again observed in the blood of the bush-buck on April 14 and 15. On the night of the 15th the antelope died; no *post-mortem* was made. Before its death, however, a monkey, a white rat, and a white mouse were inoculated with a few drops of the buck's blood.

The monkey and the white rat never became infected. The white mouse showed Spirochætes in its blood on April 20, *i.e.*, five days after inoculation. The parasites persisted in the mouse's blood for four days and then completely disappeared. The mouse recovered and lived several months without a relapse.

During the time that this mouse showed Spirochætes in its blood a second mouse was inoculated from it. Five days after the latter showed the same organisms in its blood for two days only. Subsequent daily examination for one month failed to reveal any return of the parasite.

The plate shows the appearance of the Spirochæte as seen in the blood of a white mouse. On comparing the figures of the organism as seen in the blood of a bush-buck and in that of the mouse, there is little appreciable difference to note, except that the Spirochætes in the mouse's blood appear to have fewer spirals.

It may be mentioned that no Spirochætes could be found in the fæces of the antelope under observation.

Conclusions.

1. A Spirochæte is parasitic in the blood of African antelope of the bush-buck species.
2. This organism, apparently, causes disease in the buck.
3. White mice can be infected, but the Spirochæte causes little or no ill-health, and soon disappears from the blood.

DESCRIPTION OF PLATE 15 (SPIROCHÆTES).

FIGS. 1, 2, and 3.—Spirochætes from Bushbuck. $\times 2000$.

FIGS. 4 and 5.—Spirochætes from Mouse. $\times 2000$.

38.—THE EXAMINATION OF BLOOD-SUCKING INSECTS, OTHER THAN TSETSE FLIES, FOR THE PRESENCE OF PARASITES.

Table 72.—The Examination of Freshly-caught Blood-sucking Insects, other than Tsetse Flies, for the Presence of Parasites.

Species.	No. examined.	Whence obtained.	Microscopical examination.	
			Fresh.	Stained.
<i>Hæmatopota</i>	1	Mpumu	—	Fresh mammalian blood.
"	1	"	—	—
"	1	"	—	—
<i>Tabanus virgatus</i> .	1	"	—	—
"	1	Feeding on cattle and goats.	—	—
<i>Tabanus secedens</i> .	40	" " "	—	—
<i>Chrysops</i> ...	1	" " "	—	—
Larvæ of <i>Coleoptera</i> ?	4	From the huts of natives, who say they are blood-suckers.		
"	12	" " "	—	—
"	14	" " "	—	—
Small " black beetles.	57	" " "	—	—
Larvæ ...	10	" " "	One contained bodies believed to be red corpuscles. No spirilla or other parasites.	
<i>Ornithodoros moubata</i> .	5	Unyoro		

Result.—Negative.

Table 73.—The Examination of Insects other than Tsetse Flies (after they had been fed on Infected Blood), for Developmental Forms of Trypanosomes.

Experiment No.	Species of insect.	No. used.	Variety of trypanosome.	Hours after infected feed.	Result
577	<i>Simulium danuosum</i> .	6	<i>Trypanosoma pecorum</i> .	16	Negative.
437	<i>Hæmatopota</i> ...	5	<i>Trypanosoma gambiense</i> .	18	"
451	<i>Pediculi vestimentorum</i> .	6	"	24	"
527	<i>Ornithodoros moubata</i> (young females).	2	"	24	No development or multiplication of trypanosomes.
527	"	2	"	48	"
527	" (nymphs).	2	"	48	"

Result.—Negative.

Table 74.—To Study the Fate of *Trypanosoma gambiense* in the Body of the Bed-bug (*Cimex lectularius*).

Hours after feeding.	Results of dissection of the gut.		Remarks.
	Fresh preparation.	Stained preparation.	
1	Living trypanosomes seen.	Trypanosomes show no sign of degeneration.	{ Scanty infection in the monkey on which the bugs were fed.
1½	—	—	
1½	—	—	
22½	Living trypanosomes seen.	Trypanosomes show a little vacuolation and degeneration. The flagella and undulating membranes were clear.	
23	—	Trypanosomes show degeneration.	
23½	A few trypanosomes showing feeble movement.	Quite a considerable number of healthy trypanosomes seen. One part showed about ten conglomerated, mostly with their blunt ends together, and all quite sharp and well defined.	
24	—	A few trypanosomes present, but hardly recognisable.	
24	—	—	
24	—	—	
24	—	—	
24	—	—	
24	—	—	
24	—	—	
48	—	—	
72	—	—	
72	—	—	
72	—	—	
72	—	—	
72	—	—	
72	—	—	

Remarks.—Living trypanosomes were recognised as late as 23½ hours after ingestion, and in the stained preparation a mass of parasites was seen, suggesting agglomeration. In the other experiments the trypanosomes had disappeared from the alimentary

canal of the bug in 24 hours. The bugs died too soon to permit of transmission experiments being performed satisfactorily.

- 39.—REPORT ON A COLLECTION OF BLOOD-PARASITES MADE BY THE SLEEPING SICKNESS COMMISSION, 1908-9, UGANDA. BY E. A. MINCHIN, M.A., F.R.S., PROFESSOR OF PROTOZOOLOGY IN THE UNIVERSITY OF LONDON.

This paper was published in the Reports of the Sleeping Sickness Commission, No. X., p. 73.

G. DISEASES CAUSED BY PIROPLASMS IN UGANDA.

40.—“ AMAKEBE,” A DISEASE OF CALVES IN UGANDA.

This paper was published in the ‘Proceedings’ of the Royal Society, B, vol. 82, 1910, p. 256, and reprinted in the Reports of the Sleeping Sickness Commission, No. X., p. 86.

It is concluded that the disease of calves called “Amakebe” is East Coast fever, caused by *Piroplasma parvum* and carried principally by the brown tick—*Rhipicephalus appendiculatus*.

41.—THE OCCURRENCE OF PIROPLASMOSIS IN A WHITE MOUSE.

A white mouse, one of a number of these animals obtained in Naples in September, 1908, was taken to Uganda for experimental purposes.

On April 20, 1910, the animal was inoculated with a few drops of blood from another mouse suffering from Spirochæte infection derived from the blood of a bush-buck.

On April 27, the first-named mouse, in addition to an infection with Spirochætes, was found to be suffering from piroplasmosis, as was proved by the discovery of numerous intracorpuseular parasites of the genus *Piroplasma* infecting its red-blood cells. The parasite was examined in stained preparations only, and the following examples were recognised:—

1. Pear-shaped forms occurring singly or in couples. They measure about 5 microns in length, and occupy the centre of the red cell. A small compact nucleus is placed near the thin end of the parasite. Stained with Giemsa the cytoplasm is coloured pale-blue around the periphery of the cell, and in the centre there are circular unstained areas resembling vacuoles (*see* Plate 15, p. 184, figs. 1 and 2, lower part).

2. Large and small spherical, or ring-shaped varieties, in which the centre of the organism is unstained or vacuolated and the chromatin material lumped together on one side of the periphery, recalling the familiar signet-ring phase of the malarial parasite.

3. Figs. 6 and 7, Plate 15, p. 184, show club-shaped and rod-shaped forms. Fig. 7, Plate 15, shows also a fairly common example of the parasite, viz., a minute chromatin dot situated on the margin of the red-blood corpuscle.

4. In Figs. 8, 9, and 10, Plate 15, p. 184, the parasite is seen to be multiplying by two-fold fission within the red cell.

5. Figs. 11 and 12, Plate 15, p. 184, represent large irregular-shaped parasites, one of which shows minute processes like the pseudopodia of *Amœba*.

These organisms were observed in the mouse's blood for four successive days only, and then they completely disappeared.

It is not unlikely that this *Piroplasma* is of the same species as that described by Dr. Wenyon* as infecting the blood of the common wild zebra mouse of the Sudan.

No ticks were found on the mouse. The carrier of the parasite is unknown. The animal recovered and was quite healthy when the Commission left Mpumu in August, 1910.

* “Third Report of the Wellcome Research Laboratories, Khartoum,” 1910.

H. DISEASES OF NATIVES IN UGANDA OTHER THAN SLEEPING SICKNESS.

42.—“ MUHINYO,” A DISEASE OF NATIVES IN UGANDA.*

When the Sleeping Sickness Commission passed through Kampala, the native capital of Uganda, at the end of October, 1908, on their way to their camp at Mpumu, they were informed by Sir Apolo Kagwa, K.C.M.G., the Prime Minister, that a new disease had broken out in the province of Ankole, and that many people were sick.

This is probably the same disease which was described by Dr. A. G. Bagshawe in 1906. He gives the history and symptoms of nine cases which he saw in Ankole. He concluded that the disease was beri-beri, and states that at one village 25 per cent. of the inhabitants were suffering from a more or less severe form of the disease.

In the same year Dr. L. D. Lowsley also described “Muhinyo,” but was of opinion that it might possibly be dengue with persistent joint pains.

Nothing more seems to have been written about “Muhinyo” until the beginning of 1909, when Dr. A. C. Rendle reported its presence in large numbers in the country round Lake Albert Edward. He says that all classes suffer, and that he has no hesitation in saying that “the disease is chiefly allied to kala-azar, the black death of India.”

Thanks to the kindness of Sir Apolo Kagwa and Chief Saulo Mayanja Lumama, the Commission had an opportunity of seeing a case of “Muhinyo,” which was sent to Mpumu from Ankole in January, 1909. This patient, who was said to have been ill for three months, was extremely weak and thin, but otherwise he showed no symptoms which pointed to any special disease.

As no other cases could be sent such a long journey as to Mpumu, it was decided that a member of the Commission should proceed to the district, in order to examine sick natives whom A. H. Watson, Esq., the District Commissioner, had kindly undertaken to have collected there.

On May 23rd, 1909, Dr. A. D. P. Hodges, C.M.G., the Principal Medical Officer, Uganda Protectorate, accompanied by Colonel Sir David Bruce, Director of the Commission, went to Masaka, on the borders of Ankole, where they found some fifty sufferers from this disease awaiting them.

Distribution of “Muhinyo” in Uganda.

The principal focus of the disease is along the eastern shore of Lake Albert Edward, which corresponds nearly to longitude 30° E., in the latitude of the Equator. The most severe cases have been met with at Katwe (Fort George), a settlement on the eastern shore of Lake Albert Edward. It appears to have spread down the eastern shore of this lake, and to have extended in a south-easterly direction into Ankole. Cases have been recorded as far east as the

* Reprinted from the ‘Proceedings’ of the Royal Society, B, vol. 82, 1910.

western shore of Lake Victoria, and as far north as the Katonga River, which runs parallel to, and about ten miles north of, the Equator. The disease is therefore quite limited in its distribution.

There is no evidence to show how it originated.

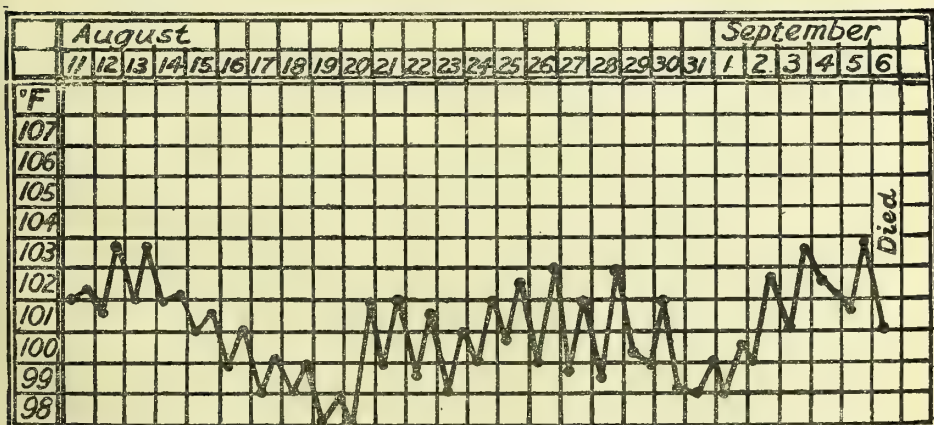
Epidemiology.

The tribes most affected by the disease are the Bakonjo and the Basongora. The former are morally and socially about the lowest class of people to be met with in Uganda. They are abjectly poor and dirty in their persons and in their habits. They live in rude grass huts, which they share with their domestic animals. The Bakonjo keep goats and, if they can afford them, cattle also. They prefer the milk of the cow, but also drink largely of goats' milk. The Basongora are a higher type of native, and resemble in appearance and customs the Bahima, the aristocracy of Uganda. They are not so poor as the Bakonjo, whom they use as serfs; they keep cattle and goats, and consume the milk of both animals. The flesh of the goat is largely eaten by both classes in a partially-cooked state. The milk of sheep is occasionally used in default of that of the other animals.

Clinical Symptoms of “ Muhinyo.”

As the result of the examination of the fifty cases sent into Masaka, it appeared that the principal symptoms of “ Muhinyo ” are fever, profuse sweating, pains in the joints and along the course of nerves, swelling of the various joints, especially the ankles, and extreme weakness and emaciation. The disease is of long duration; most of the patients have been ill for several months. In 13 cases, taken at random, the average duration was three and a-half months. Another had been ill for two years.

The following temperature chart was the only one which could be obtained.



In most cases there was no marked enlargement of the spleen or liver, nor symptoms of paresis or paralysis. The microscopical examination of the blood showed various degrees of anæmia, but no parasites or marked changes in the white blood corpuscles could be detected. Further, the examination of the splenic pulp, obtained by puncture of the spleen, failed to reveal the presence

of the parasites of kala-azar. It was therefore evident that " Muhinyo " was neither kala-azar nor beri-beri, but the long duration of the fever, the joint pains, and the extreme weakness and emaciation suggested a continued fever, such as typhoid or Malta fever.

Examination of the Blood for Agglutinative Phenomena.

The blood of several of these cases was therefore tested with *Bacillus typhosus* and *Micrococcus melitensis* by Widal's method, with the result that no reaction was obtained with the former, but positive results, in fairly high dilutions, were got with the latter.

The following table represents the result of the examination of the blood of " Muhinyo " with a strain of *M. melitensis* from Malta, from which it will be seen that five out of the seven cases examined gave a positive reaction:—

Table 75.

Number of experiment.	Dilution of serum.			Control.
	1 in 50.	1 in 100.	1 in 200.	
927	+	+	—	—
928	+	+	—	—
929	—	—	—	—
930	—	—	—	—
931	+	+	+	—
932	+	+	+	—
933	+	+	—	—

Isolation of the Micrococcus of Malta Fever from the Spleens of Cases of " Muhinyo."

The next thing to be undertaken was the isolation of the *M. melitensis* from the tissues of patients suffering from " Muhinyo."

The spleens of two cases (925 and 926) were punctured in the usual way, and the splenic pulp smeared on the surface of tubes of nutrient agar-jelly. Small white colonies were grown from both cases, and these were sub-cultured and used to study the morphology, cultural characters, and animal reactions of the organism of which they were composed.

Morphology.—Under the microscope the organisms were found to be minute micrococci, indistinguishable in size or appearance from the *M. melitensis*.

Cultural Characters.—In the same way it was found that the sub-cultures of the organism showed after some days as minute transparent colonies, resembling tiny drops of dew, which afterwards became more opaque, and in no way differed from colonies of *M. melitensis* cultivated under the same circumstances.

Animal Reactions.—The sub-cultures were also emulsified in saline solution and injected into a monkey and rabbit. The monkey sickened with fever, and when the agglutinating power of its blood was tested with the strain of *M. melitensis* from Malta it was found to give a complete reaction in a dilution of 1 in 200. Having thus proved that two animals treated with the "Muhinyo" organism gave a serum capable of agglutinating a known *M. melitensis* from Malta, the converse experiment was made.

A rabbit was inoculated with the Malta strain, and its serum tested on the "Muhinyo" organism. This rabbit's serum, immunised against Malta Fever, agglutinated the "Muhinyo" organism in a dilution of 1 in 200; and thus the proof that the micrococcus obtained from the spleen of "Muhinyo" cases and that obtained from cases of Malta fever were identical was established.

Examination of Goats from the "Muhinyo" District, to ascertain if they are Reservoirs of the Virus of Malta Fever.

By Widal's Reaction.—In Malta the Royal Society Commission discovered, in 1905, that the drinking of goats' milk was the sole mode of infection in Malta fever. Many of the Maltese goats were examined, and 50 per cent. of them found to be affected in some way by the disease, while 10 per cent. were actually excreting the *M. melitensis* in their milk.

It was therefore a matter of importance, as well as curiosity, to ascertain if the Ankole goats also suffered from Malta fever, and if the causation of this disease was the same in Central Africa as it had been proved to be on the shores of the Mediterranean, in the Soudan, and in South Africa.

When Sir Apolo Kagwa was approached as to the feasibility of obtaining goats from the most affected districts, he informed the Commission that he would see what could be done. About six weeks later a flock of goats, numbering in all twenty-four, was driven up to the laboratory at Mpumu, and it was stated that these had come from a place where "Muhinyo" was common. They were at once examined, with the result that the blood of three out of their number reacted to the strain of *M. melitensis* obtained from cases of "Muhinyo," and also to the Malta strain.

The following tables give the details:—

Table 76.—*Micrococcus melitensis* ("Muhinyo" Strain).

No. of experiment.	Dilutions of serum.					Control.
	1 in 10.	1 in 20.	1 in 50.	1 in 100.	1 in 200.	
1512	+	+	+	—	...	—
1507	+	+	+	+	...	—
1776	+	+	+	+	+	—

Table 77.—*Micrococcus melitensis* (Malta Strain).

No. of experiment.	Dilutions of serum.				Control.
	1 to 10.	1 to 20.	1 to 50.	1 to 100.	
1512	+	+	+	—	—
1507	+	+	+	+	—

Isolation of the Micrococcus of Malta Fever from the Tissues of the Goats.—After having found that some of the Ankole goats reacted to the agglutination test, an attempt was made to isolate the *M. melitensis* from their tissues. This proved successful in two cases. The following experiment gives one of these in detail:—

Experiment 1475. Goat.

August 11th, 1909.—This goat, which was one of a herd from Ankole, died this morning. The spleen was removed, and small portions of the pulp spread over the surface of agar tubes.

August 16th.—A growth consisting of several very small, round, white colonies appeared after three days. A stained preparation from one of these showed that they were composed of organisms resembling *M. melitensis*. Sub-cultures made.

September 29th.—The growth from two agar tubes was made into an emulsion with salt solution, and an agglutination test made with serum from a rabbit immunised against *M. melitensis*, Malta strain.

The result was that the organism from the goats agglutinated completely in a dilution of 1 in 100, and the proof was complete that the Ankole goats are liable to contract Malta fever, and to act as a reservoir of the virus.

Conclusions.

- (1.) " Muhinyo " is Malta fever.
- (2.) " Muhinyo " is conveyed from the goat to man by the drinking of goats' milk.

I. DISTRIBUTION OF BITING FLIES AND TICKS IN UGANDA.

43.—DISTRIBUTION OF BITING FLIES IN UGANDA.

Introduction.

In 1903, when it was desired by the Sleeping Sickness Commission to work out the distribution of *Glossina palpalis*, in order to ascertain if it coincided with the distribution of Sleeping Sickness, specimens of the fly and the following letter was sent to every official and missionary in Uganda:—

“ ‘Sleeping Sickness’ in many ways is very similar to the ‘fly disease’ of South Africa. It is caused by the same kind of parasite, and is possibly carried from man to man by some insect, as the ‘fly disease’ is carried by the tsetse fly (*Glossina morsitans*). A species of tsetse fly (*Glossina palpalis*) is also found in Uganda, especially along the shores of the Lake and islands. It is possible that this fly acts as the carrier of the infective agent of Sleeping Sickness. If this is so, the disease can only occur in places where the fly is found. In other words, no tsetse fly, no Sleeping Sickness.

“ In order to settle this question the distribution of this fly must be carefully worked out. To assist in this would you kindly send collections of biting flies from your district?

“ It is important that the position of the place where these flies are found should be laid down. This can be done by giving the latitude and longitude in degrees and minutes, or by saying how many miles it is from a known place in a westerly or south-south-westerly, &c., direction. Still better would it be to take a tracing from the map of the district, and on this to plot out the distribution of the fly and of the sickness.”

A memorandum,* asking that biting flies, mosquitoes, &c., might be sent to the laboratory at Mpumu, was distributed in Uganda by the Commission in 1908.

During June, July and August, 1903, some 460 collections of biting flies were sent in from all parts of Uganda.

Under the term Uganda are included several Provinces—that of Uganda proper, Usoga, Unyoro, Toro and Ankole. Of these, Uganda proper is the most thickly populated and the most civilised.

The biting flies, on arrival in the laboratory at Entebbe, were at once sorted out, and the different kinds sent, from time to time, to Mr. Austen, of the British Museum, who named them.

In the same way, the Sleeping Sickness Commission in 1908-9-10 dealt with all the biting flies they collected or received.

The bulk of this material was obtained by the natives themselves, who collected all the flies they could lay their hands on,

* See Appendix IV.

and forwarded them to the laboratory, packed, as a rule, in an empty gourd, or, at other times, merely wrapped in a banana leaf. Many were, therefore, in a damaged condition, and must have given Mr. Austen much trouble to name.

This, then, is the origin of the collection of biting flies which forms the subject of this report.

It will be readily understood that the number of biting flies collected in any given place, depended partly on the density of population of the district and on the energy of the chief, or official, who undertook to make the collection. If the district was sparsely populated, few collections would be sent in. If the headman took little interest in the matter, little would be done by his subordinates. Naturally, a large number of the flies came from the Province of Uganda, since the population is large and the natives are well under the control of their chiefs. The richness of the collections from the district of Usoga was due to the energy and the interest displayed in the matter by the then Provincial Commissioner, Mr. William Grant. This gentleman was famous for his knowledge of, and his influence over, the natives of his Province.

The dotted maps representing the distribution of the biting flies are, therefore, deceptive. Take, for example, *Tabanus thoracinus*. Here the hand of Mr. Grant is seen, since there has been sent from Usoga some 80 collections of flies in which this species was found. Twenty-nine collections have been sent in from Uganda, three from Unyoro, five from Toro, and only one from Ankole. In spite of the map, it is quite possible this fly is as common in Ankole as in Usoga. Again, the dots give no hint as to the numbers of the fly found. If a thousand *Tabanus thoracinus* came from a particular place, and only one *Tabanus fasciatus*, the same-sized dot would be placed on each map. Also there is no record of the seasonal occurrence of these species. In many places the collecting would go on for a week or two only, and only those species occurring at the time would come in for notice.

It is evident, then, that to get a fairly complete knowledge of the distribution of these biting flies, it would be necessary to collect them in many places and at all seasons of the year.

Our object in trying to mark out the distribution of the various biting flies, was to find out if any of them act as carriers of disease. For example, by marking out the distribution of *Glossina palpalis* in Uganda, it became at once manifest that this fly was the carrier of Sleeping Sickness (*Trypanosoma gambiense*).

The chief trypanosome diseases in Uganda, besides Sleeping Sickness, are caused by *Trypanosoma pecorum*, *brucei*, *vivax*, *uniforme* and *nanum*. Now we know that *Trypanosoma brucei* is carried by *Glossina morsitans* and *pallidipes*; but if this disease occurs outside the range of distribution of these two tsetse flies, then there must be another carrier. If, on the other hand, the disease caused by *Trypanosoma brucei* is only found where *morsitans* and *pallidipes* are found, this knowledge should be of use in evading this disease.

We know that *Trypanosoma vivax* is carried by *Glossina palpalis*. If it is proved that it is only carried by this fly, then it should be easy to escape it. When the Government transport cattle in Kampala, in 1909, were dying rapidly from this *Trypanosoma vivax* disease, the simple removal of their kraal from the vicinity of the Lake-shore and *Glossina palpalis* at once put an end to the epidemic.

So in the same way it would be useful to find out the carriers of *Trypanosoma pecorum* and *Trypanosoma nanum*; and to do this a careful mapping out of the distribution of these diseases and of the various biting flies seems to us to be the first step. This will doubtless take time and labour to accomplish, but it is necessary work if any attempt is to be made to control the diseases of stock in Uganda.

The blood-sucking diptera of Uganda included in this report belong to the families *Tabanidæ*, *Muscidæ*, *Simuliidæ*, *Chironomidæ* and *Hippoboscidæ*. The mosquitoes (*Culicidæ*) and sand-flies (*Psychodidæ*) are not included.

Figures of many of these flies are given in Mr. Austen's book on "African Blood-sucking Flies," 1909, published by the British Museum (Natural History), Cromwell Road, London, S.W.

Twenty-three species of *Tabanus*, 16 *Hæmatopota*, four *Chrysops*, one *Rhinomyza*, two *Stomoxys*, four *Glossina*, one *Simulium*, two *Culicoides*, and one *Echestypus* are included in this list.

The species *Auchmeromyia luteola* (Fabricius) is not included in this list as it is not a blood-sucker. The interest of this species lies not in the perfect insect but in the maggot, which lives in the floor of native huts and crawls out at night to suck the blood of human beings whilst they sleep. The maggots are of the usual whitish colour, but after a meal of blood become red. Their habits and morphology were described by Drs. Dutton, Todd and Christy, in *Memoir XIII.* of the Liverpool School of Tropical Medicine, 1904.

The following is a list of the species mentioned in this report:—

- Tabanus thoracinus* (Palisot de Beauvois).
- Tabanus obscurissimus* (Ricardo).
- Tabanus par* (Walker).
- Tabanus fasciatus* (Fabricius).
- Tabanus fasciatus niloticus* (Austen).
- Tabanus brucei* (Ricardo).
- Tabanus africanus* (G. R. Gray).
- Tabanus leucaspis* v.d. Wulp (nec Wiedemann).
- Tabanus biguttatus* (Wiedemann).
- Tabanus maculatissimus* (Macquart).
- Tabanus brumpti* (Surcouf).
- Tabanus secedens* (Walker).
- Tabanus socialis* (Walker).
- Tabanus tæniola* (Palisot de Beauvois).
- Tabanus tæniola form variatus* (Walker).
- Tabanus gratus* (Loew).

Tabanus variabilis (Loew).
Tabanus ditæniatus (Macquart).
Tabanus ruficrus (Palisot de Beauvois).
Tabanus fuscomarginatus (Ricardo).
Tabanus albipalpus (Walker).
Tabanus sp. nov., near *T. insignis* (Loew).
Tabanus irroratus (Surcouf).
Hæmatopota vittata (Loew), syn. *H. pulchrithorax* (Austen).
Hæmatopota ugandæ (Ricardo).
Hæmatopota brucei (Austen).
Hæmatopota inornata (Austen).
Hæmatopota mactans (Austen).
Hæmatopota sp. nov.
Hæmatopota decora (Walker).
Hæmatopota fusca (Austen).
Hæmatopota sp. nov.
Hæmatopota hirta (Ricardo).
Hæmatopota tenuis (Austen).
Hæmatopota brunnescens (Ricardo).
Hæmatopota nigrescens (Ricardo).
Hæmatopota denshamii (Austen).
Hæmatopota similis (Ricardo).
Hæmatopota unicolor (Ricardo).
Chrysops brucei (Austen).
Chrysops distinctipennis (Austen).
Chrysops funebris (Austen).
Chrysops centurionis (Austen).
Rhinomyza perpulcra (Austen).
Stomoxys nigra (Macquart).
Stomoxys calcitrans (Linnæus).
Glossina palpalis (Rob.-Desv.).
Glossina morsitans (Westwood).
Glossina pallidipes (Austen).
Glossina fusca (Walker).
Simulium damnosum (Theobald).
Culicoides brucei (Austen).
Culicoides milnei (Austen).
Echestypus paradoxus (Newstead).

FAMILY: TABANIDÆ.*

GENUS: TABANUS.

Tabanus thoracinus (Palisot de Beauvois).

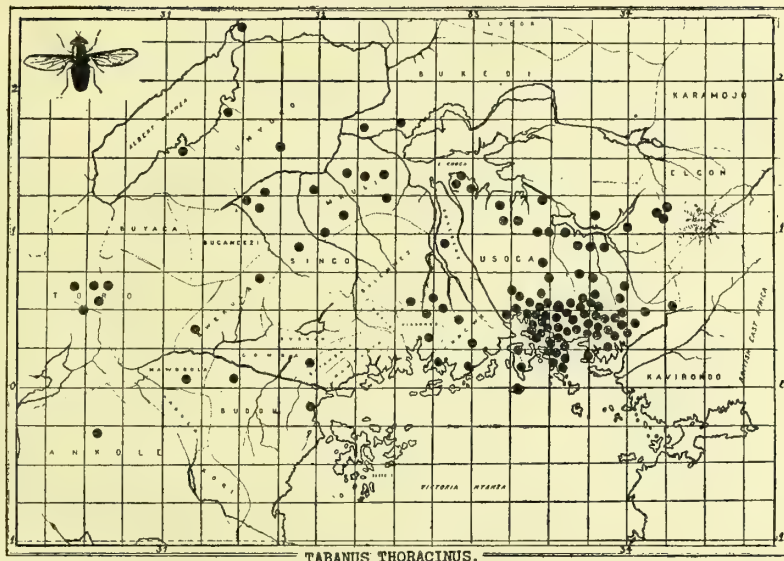
(Plate V., 38, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—Widely spread throughout tropical Africa.

Distribution in Uganda.—Map 1 shows that *Tabanus thoracinus* is met with in most parts of Uganda; it was found in most of the areas where fly-catching operations were carried out.

* The native name of all the *Tabanidæ* is "Dumambogo," singular; "Malumambogo," plural.

Map 1.



The following table gives some information about the various specimens which were sent to the Commission, 1908-9-10, for identification:—

Table 78.—*Tabanus thoracinus*.

Date.	Locality.	District.	Province.	Lat.*	Long.*	Donor.	Remarks.
1908.							
Dec. 8	Viatente ...	—	—	0 1	0 1	Ham Mukasa, Sekibobo.	
Dec. 8	Mpumu ...	Chagwe ...	Uganda	0 15 N.	32 50 E.	Commission.	
1909.							
Feb. 1	Wajalulu	Sebondi's	Usoga ...	1 0 N.	33 40 E.	Capt. H. M. Tufnell.	
Mar. 18	Fort Portal	—	Toro ...	0 40 N.	30 15 E.	Dr. A. Bond ...	Caught on cattle.
Mar. 21	Jinja ...	—	Usoga ...	0 30 N.	33 15 E.	Capt. H. M. Tufnell.	
May 6	Sio river ...	Buoni ...	"	0 30 N.	34 55 E.	Capt. A. E. Hamerton.	
May 15	Kampala...	Kiadondo	Uganda	0 20 N.	32 35 E.	Sir Apolo Kagwa.	
June 17	Koba ...	—	Nile ...	2 30 N.	31 40 E.	Dr. C. Wiggins.	
July 1	Daudi ...	Fort Portal	Toro ...	0 40 N.	30 20 E.	Dr. A. Bond.	
July 1	Khagora ...	"	"	0 40 N.	30 18 E.	"	
July 1	Fort Portal	—	"	0 40 N.	30 15 E.	"	
July 1	Forest ...	Fort Portal	"	0 40 N.	30 15 E.	"	
July 26	Nagongera	Budama...	—	0 40 N.	34 0 E.	P. W. Ferryman.	Biting a mule.
July 26	Mpologoma	"	—	1 0 N.	33 45 E.	"	
July 26	Bunyali ...	"	—	0 45 N.	33 44 E.	"	
July 26	Butelein ...	"	—	1 5 N.	33 49 E.	"	
Aug. 4	Kampala...	Kiadondo	Uganda	0 20 N.	32 35 E.	Director of Transport.	
Aug. 7	Masaka ...	Buddu ...	"	0 20 S.	31 50 E.	D. L. Baines.	
Aug. 15	Kampala...	Kiadondo	"	0 20 N.	32 35 E.	Director of Transport.	On many occasions.
Aug. 18	Kabula- Muliro.	Bulemezi	"	0 40 N.	32 18 E.	Capt. F. P. Mackie.	
Sept. 4	Jinja ...	—	Usoga...	0 30 N.	33 15 E.	Dr. H. B. Owen.	
Sept. 3	Bussu ...	—	"	0 30 N.	33 20 E.	Dr. H. Bayon.	
Oct. 14	Jinja ...	—	"	0 30 N.	33 15 E.	Capt. G. Lane.	
1910.							
May 12	Mpumu ...	Chagwe ...	Uganda	0 15 N.	32 50 E.	Commission.	

* The degrees of latitude and longitude, as regards locality, are approximate only.

Description of the Fly.—It is a medium-sized *Tabanus*, with an orange abdomen, a grey thorax, and semi-transparent wings. The eyes in the living condition are of a beautiful green colour.

Bionomics.—This fly attacks man and cattle.

Tabanus obscurissimus (Ricardo).

(Plate V., 37, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—This species has a wide range in West Africa.

Distribution in Uganda.—It was found in numbers at the Kasala stream, at the foot of Mpumu, Chagwe, Uganda ($0^{\circ} 15' N.$, $32^{\circ} 50' E.$).

Description of the Fly.—This is a dark, dusky looking tabanid, of medium size.

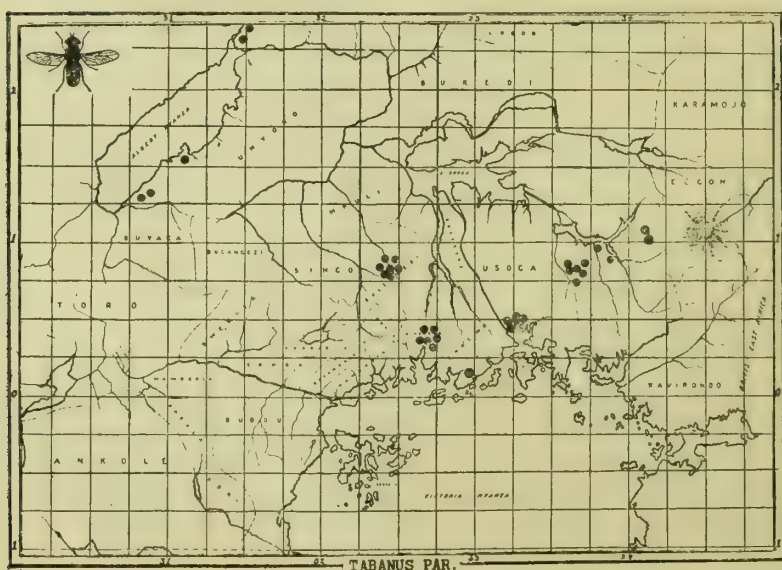
Tabanus par (Walker).

(Plate V., 39, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—One of the most widely distributed of African *Tabanidæ*.

Distribution in Uganda.—This species was received from various parts of the country, but in small numbers.

Map 2.



The following table gives in detail the locality where this fly was found and the name of the collector:—

Table 79.—*Tabanus par.*

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° ' "	° ' "	
June 17	Koba ...	—	Nile ...	2 30 N.	31 40 E.	Dr. C. Wiggins.
July 24	Tonya ...	Bugoma	Unyoro	1 20 N.	31 0 E.	Dr. J. H. Goodliffe.
" 26	Nagongera	Budama	—	0 40 N.	34 0 E.	P. W. Perryman.
" 26	Mulanda	"	—	0 37 N.	34 0 E.	"
" 26	Peta ...	"	—	0 43 N.	33 58 E.	"
" 26	—	"	—	—	—	"
" 26	—	"	—	—	—	"
" 26	Mpologoma.	"	—	1 0 N.	33 45 E.	"
Aug. 4	Kampala	Kiadondo	Uganda	0 20 N.	32 35 E.	Director of Transport.
" 12	Kumi ...	—	Bukedi	1 30 N.	33 20 E.	E. G. Morris.
" 25	K a b u l a Muliro.	Bulemezi	Uganda	0 40 N.	32 18 E.	Capt. F. P. Mackie.
Sept. 4	Jinja ...	—	Usoga	0 30 N.	33 15 E.	Dr. H. B. Owen.
1910.						
May 12	Mpumu...	Chagwe...	Uganda	0 15 N.	32 50 E.	Commission.

Description of the Fly.—It closely resembles *Tabanus thoracicus* in colouring, differing from it only in being smaller and some slight morphological details, for which the reader is referred to Mr. Austen's book on "African Blood-sucking Flies," published by the British Museum (Natural History), Cromwell Road, London, S.W.

Bionomics.—This fly readily attacks man as well as domestic animals.

Tabanus fasciatus (Fabricius).

(Plate VI., 40, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—This fly is common in West Africa, but appears to be met with across the Continent to Uganda and the Anglo-Egyptian Sudan.

Distribution in Uganda.—A considerable number of these flies was collected by the Sleeping Sickness Commission in 1903, and a few more were added to the collection during the stay of the present Commission. Of the latter, some were caught by Dr. A. Bond at Fort Portal, in Toro (0° 40' N., 30° 15' E.), whilst biting

Distribution in Uganda.—Table 80.—*Tabanus fasciatus niloticus*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° ' /	° ' /	
July 26	Nagongera	Budama	—	0 40 N.	34 0 E.	P. W. Perryman.
Aug. 30	Busoa ...	Bulemezi	Uganda	0 41 N.	32 22 E.	Capt. A. E. Hamerton.
Oct. 14	Keringia	Jinja ...	Usoga	0 32 N.	33 14 E.	Capt. G. Lane
1910.						
Mar. 1	Kibanga	Chagwe	Uganda	0 15 N.	32 50 E.	Commission.

Description of the Fly.—It resembles *Tabanus fasciatus* (Fabricius), but differs from it in some minor details, for a description of which the reader is referred to Mr. Austen's Monograph.

Bionomics.—Bites man and animals, and often produces local suppuration in the former.

Tabanus brucei (Ricardo).

(Plate VI., 41, Austen's "African Blood-sucking Flies.")

This handsome fly was found in 1903, but no specimens of it were obtained by the present Commission. It has only been found in Uganda and in the Congo. The Uganda specimens came from Ankole, and Masaka, Buddu, the former sent by Mr. Dashwood, the latter by the late Mr. Ormsby.

Tabanus africanus (G. R. Gray).

(Plate VI., 42, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—It is met with in Natal, the East Africa Protectorate, and in the Sudan as far west as Lake Chad.

Distribution in Uganda.—A few specimens were sent in, as the following record shows:—

Table 81.—*Tabanus africanus*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° ' /	° ' /	
Feb. 1	Gandinera	Sebondi's	Usoga	0 57 N.	34 0 E.	A. G. Boyle.
Mar. 21	—	Jinja ...	"	0 30 N.	32 0 E.	Capt. H. M. Tuffnell.
July 26	Nagongera	Budama	—	0 40 N.	34 0 E.	P. W. Perryman.
—	Mulanda	"	—	0 37 N.	34 0 E.	" "
—	Peta ...	"	—	0 43 N.	33 58 E.	" "
1910.						
Apr. 1	Mpumu ...	Chagwe.	Uganda	0 15 N.	32 50 E.	Commission.

Description of the Fly.—A handsome and easily recognised fly of large size and of golden-brown colour. The wing tips and two spots are transparent, but the rest of the wings are of a dark brown colour.

Bionomics.—Its bite is said to be very severe, and in man is apt to be followed by fever.

Tabanus leucaspis, v.d. Wulp (nec Wiedemann).

Geographical Distribution.—This species appears to extend in a belt across the equatorial part of Africa—from the West Coast to Uganda and the Nyasaland Protectorate.

Distribution in Uganda.—Only three specimens were sent in, two from Usoga, September 17, 1903, and the other on May 29, 1909, from the Sesse Islands (0° 10' S., 32° 10' E.) by Captain A. D. Fraser, R.A.M.C.

Description of the Fly.—The fly is easily distinguishable from the closely allied West African *T. plato* (Walk.), by the fact that the large chrome-yellow or sulphur-yellow patch on each side of the abdomen terminates abruptly on the hind margin of the third segment, instead of extending on to the fourth.

Tabanus biguttatus (Wiedemann).

(Plate VI., 44, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—According to Austen this fly is met with from Cape Colony to the Anglo-Egyptian Sudan and across Africa as far west as Lake Chad and Senegal.

Distribution in Uganda.—

Table 82.—*Tabanus biguttatus*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° /	° /	
Feb. 1	Muhina...	Sebondo's	Usoga	0 54 N.	33 58 E.	A. G. Boyle.
"	Gandinera	"	"	0 57 N.	34 0 E.	
June 17	Koba ...	"	Nile ...	2 30 N.	31 40 E.	Dr. C. Wiggins.
July 26	Mpologoma.	Budama.	—	1 0 N.	33 45 E.	P. W. Perryman.

Description of the Fly.—This is a large fly of smoky-brown colour. There is marked sexual dimorphism, the female being larger than the male. The male carries two small pyramidal yellow marks on the dorsal surface of the mid-abdominal segments,

which are not generally present in the female. The dorsal surface of the thorax of the female is pale-yellow, but that of the male is of dark smoky colour. Wings smoky-brown with clear tips.

Bionomics.—The fly attacks man, inflicting a severe bite.*

Tabanus maculatissimus (Macquart).

(Plate VI., 46, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—Found from Cape Colony to Nyasaland and the Congo Free State.

Distribution in Uganda.—This species was collected by the Commission at Kabula Muliro, in Bulemezi, and on the Kasala Stream, Mpumu, Chagwe, Uganda.

Tabanus brumpti (Surcouf).

(Plate VII., 49, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—This fly appears to have a somewhat limited distribution in equatorial Africa.

Distribution in Uganda.—

Table 83.—*Tabanus brumpti*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° /	° /	
July 26	Bunyali	Budama..	—	0 45 N.	33 44 E.	P. W. Perryman.
—	Butelein	"	—	1 5 N.	33 49 E.	"
Aug. 12	Budomero	Kumi ...	Bukedi	1 25 N.	33 20 E.	E. G. Morris.
" 20	Buzuge...	Sebondi's	Usoga	1 0 N.	33 30 E.	F. M. Isemonger.

Description of the Fly.—This is a large sized tabanid, with a steel-blue body and semi-transparent wings.

Tabanus secedens (Walker).

(Plate VII., 54, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—This species is extremely common in West Africa, and its range is known to extend from Senegal to the Congo Free State and Uganda.

* Some interesting experiments, dealing with the breeding of this fly, have been carried out by H. H. King, and are detailed in the *Third Report of the Wellcome Research Laboratories, Khartoum, 1909*, to which those interested are referred.

Distribution in Uganda.—Table 84.—*Tabanus secedens*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1908, Dec. 17	—	Bululu ...	Bukedi	1° 40' N.	33° 15' E.	Sir Apolo Kagwa	
1909, July 14	Wamula...	Singo ...	Uganda	0 25' N.	32 0' E.	"	
Sept. 24	Mpumu ...	Chagwe ...	"	0 15' N.	32 50' E.	Commission ...	West of hill.
Oct. 20	" ...	" ...	"	0 15' N.	32 50' E.	"	East of hill.
" 29	Kampala	Kiadondo	"	0 20' N.	32 35' E.	Director of Transport.	
1910, July 13	Mpumu ...	Chagwe ...	"	0 15' N.	32 50' E.	Commission ...	

Description of the Fly.—This is a medium-sized *Tabanus*, with a golden-brown body, with light gold markings. The wings are almost clear.

Bionomics.—This fly was absent from the locality of Mpumu, the seat of the Commission's laboratory, till September 24, 1909, when it appeared in large numbers on the west side of the hill. About three weeks later it appeared in equally large numbers on the east side of the hill. Almost immediately afterwards an epidemic of *Trypanosoma pecorum* disease broke out amongst the cattle on the west side of the hill, which quickly spread to those on the east side. Whether this was a mere coincidence or not it is impossible at present to say.

Tabanus socialis (Walker).

(Plate VIII., 57, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—It is met with from the Gambia to Uganda.

Distribution in Uganda.—On March 14, 1909, specimens were sent by E. Brown, Esq., from the Mabira Forest in Chagwe. He says: "It has appeared only the last week, and specimens of it were brought to me by a herdsman who said that it carried cattle sickness. The natives agree that it turned up only last week; now there are hundreds here even in the houses." One specimen was also sent by T. Grant, Esq., on March 7, 1909, who obtained it from the Ngussi River in Bugoma, Unyoro (1° 15' N., 30° 48' E.). It was also found by the Commission at Mpumu.

Description of the Fly.—A medium to large-sized tabanid, without very marked peculiarities.

Bionomics.—It bites man and domestic animals as well as game.

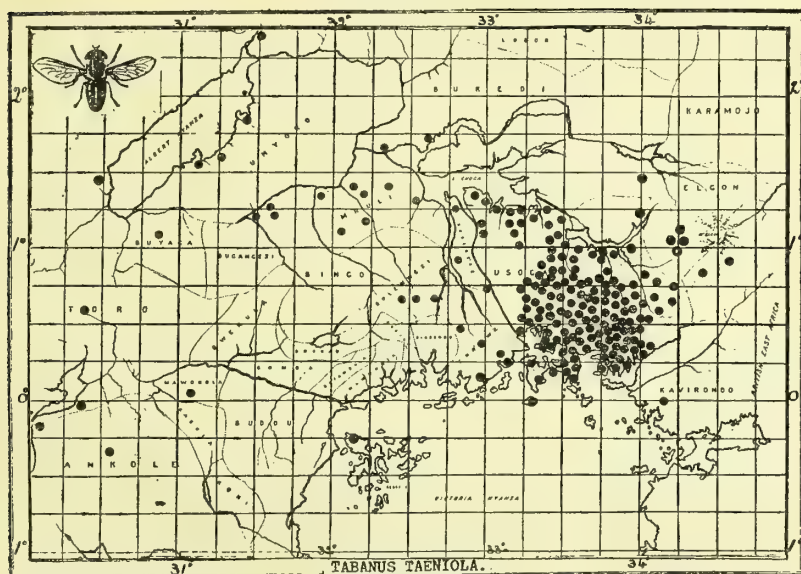
Tabanus tæniola (Palisot de Beauvois).

(Plate VIII., 61, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—According to Austen, "there can be no doubt whatever that it is the most widely-distributed of the African *Tabanidæ*."

Distribution in Uganda.—The following map represents the distribution of this species in Uganda.

Map 4.

Table 85.—*Tabanus taeniola*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1909.							
Feb. 1	Gandinera	Sebondi's	Usoga ...	0 57 N.	34 0 E.	A. G. Boyle.	On cattle.
" 17	Namukekera.	Bulemezi	Uganda	0 45 N.	32 30 E.	L. C. Lea-Wilson.	
" 22	Myanja river.	"	"	0 50 N.	32 20 E.	Capt. Bateman and Mackie.	
Mar. 18	Fort Portal	—	Toro ...	0 40 N.	30 15 E.	Dr. A. Bond ...	
" 21	Jinja ...	—	Usoga ...	0 30 N.	33 15 E.	Capt. H. M. Tufnell.	
Apr. 7	Fort Portal	—	Toro ...	0 40 N.	30 15 E.	Dr. A. Bond ...	
May 6	Wakoli's	—	Usoga ...	0 30 N.	34 40 E.	Capt. Hamerton.	
" 27	Kakoi river.	Bulegga ...	West of Lake Albert.	1 30 N.	30 15 E.	T. Grant.	
" 29	Sesse ...	Lake Victoria.	—	0 10 S.	32 10 E.	Capt. A. D. Fraser.	
June 17	Koba ...	—	Nile ...	2 30 N.	31 40 E.	Dr. C. Wiggins.	
July 26	Nagongera	Budama ...	—	0 40 N.	34 0 E.	P. W. Perryman.	Bites cattle and antelope.
" 26	Bunyali ...	—	—	0 45 N.	33 44 E.	"	
" 27	Namukekera.	Bulemezi	Uganda	0 45 N.	32 30 E.	L. C. Lea-Wilson.	
Aug. 4	Kampala	Kiadondo	"	0 20 N.	32 35 E.	Director of Transport.	
" 6	Namukekera.	Bulemezi	"	0 45 N.	32 30 E.	L. C. Lea-Wilson.	
" 12	Bulangi ...	Wakoli's	Usoga ...	0 30 N.	33 50 E.	E. G. Morris.	
Sept. 24	Lake Rubi	—	Nile ...	3 0 N.?	31 30 E.	Commander H. Hutchinson.	
Oct. 12	Lake Gedge	Karamoja	Bukedi	1 40 N.	34 15 E.	E. G. Morris.	
" 14	Mpumu ...	Chagwe ...	Uganda	0 15 N.	32 50 E.	Commission.	
1910.							
July 13	"	" ...	"	0 15 N.	32 50 E.	"	

Description of the Fly.—It is a medium-sized tabanid with almost transparent grey wings. The body is reddish-brown and carries a stripe or row of dots of varying pattern down the middle of the dorsal surface.

Bionomics.—It bites human beings and animals, and gives rise to much irritation.

Tabanus variatus (Walker).*

(Plate VIII., 59, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—It is met with almost as widely as the former species, *i.e.*, practically from Cape Colony to the Sudan and Abyssinia.

Distribution in Uganda.—The map shows the general distribution at a glance, whilst the following columns fill in the details.

Map 5.

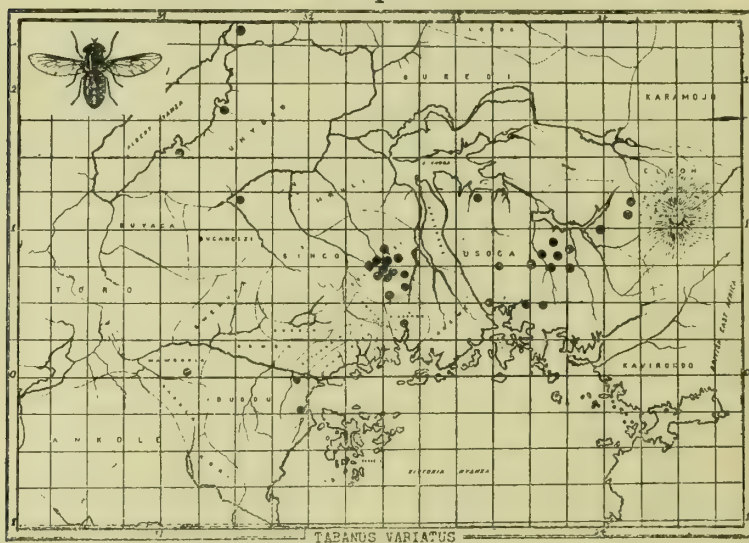


Table 86.—*Tabanus variatus*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1909.							
July 26	Nagongera	Budama ...	—	0° 40' N.	34° 0' E.	P. W. Perryman.	
" 26	Mulanda	" ...	—	0° 37' N.	34° 0' E.	"	
" 26	Peta	" ...	—	0° 43' N.	33° 58' E.	"	On cattle.
" 26	Bunyali.	" ...	—	0° 45' N.	33° 44' E.	"	
" 26	Butelein	" ...	—	1° 5' N.	33° 49' E.	"	
" 28	Namukera	Bulemezi	Uganda	0° 45' N.	32° 30' E.	L. C. Lea-Wilson.	
" 31	"	"	"	0° 45' N.	32° 30' E.	"	
Aug. 2	"	"	"	0° 45' N.	32° 30' E.	"	
" 6	"	"	"	0° 45' N.	32° 30' E.	"	
" 12	Musitwa...	Wakoli's	Usoga ...	0° 30' N.	34° 15' E.	E. G. Morris.	
" 18	Kabula	Bulemezi	Uganda	0° 40' N.	32° 18' E.	Capt. F. P. Mackie.	Open country; veldt; transport kraal; fords; game track; swamps; river side.
Sept. 4	Jinja ...	—	Usoga ...	0° 30' N.	33° 15' E.	Dr. H. B. Owen.	

* *T. variatus* (Walk.) is merely a form of *T. tæniola* (Pal. de Beauv.).

Description of the Fly.—A medium to large-sized tabanid, with clear wings. The prevailing colour of the dorsal surface of the thorax is grey, whilst that of the abdomen is brownish-red. On the latter surface a series of arrow-head marks of lighter colour occupy the middle line with the arrow points directed anteriorly.

Bionomics.—This fly is a voracious blood-sucker, selecting cattle and other domestic animals in preference to man.

Tabanus gratus (Loew).

(Plate X., 76, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—Specimens of this fly have been obtained from Nyasaland, Uganda, the Nile Province, and Northern Nigeria.

Distribution in Uganda.—Specimens have been taken on the Victoria Nile by Major E. D. W. Greig, I.M.S., by the late Dr. Densham when travelling in the Nile Province, and at Jinja by Dr. H. B. Owen.

Description of the Fly.—This is a small fly with transparent wings, a grey thorax and a brown abdomen, which shows three longitudinal white stripes on its dorsal aspect. The eyes (according to the late Dr. Densham, quoted by Austen) are "in the living insect, a beautiful greenish-blue, with horizontal zigzag bands of crimson purple."

Bionomics.—Its blood-sucking habits are similar to others of its genus.

Tabanus variabilis (Loew).

(Plate X., 78, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—This species has been met with in Cape Colony, in Uganda, and in the Congo Free State.

Distribution in Uganda.—Four specimens were forwarded to and collected by the present Commission, as follows:—

Table 87.—*Tabanus variabilis*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909. July 26	Bunyali	Budama	—	0° 45' N.	33° 44' E.	P. W. Perryman.
Oct. 29	Kampala	Kiadondo	Uganda	0 20 N.	32 35 E.	Director of Transport.
" 30	Damba Island	Lake Victoria	—	0 0	32 45 E.	Capt. A. D. Fraser.
1910. Mar. 10	Mpumu	Chagwe...	Uganda	0 15 N.	32 50 E.	Commission.

Description of the Fly.—This is a species of *Tabanus* with clear wings and a greyish-brown body. The dorsal surface of the abdomen shows darker markings down the middle line.

Bionomics.—Bites domestic animals and game.

Description of the Fly.—This fly is one of the largest of the African *Tabanidae*, but without very marked colouring. Its body is uniformly steely-blue, and the wings dark but clear and without defined markings.

Bionomics.—The native herdsmen informed Mr. Leakey that the fly was called the “biter of buffalo,” because some years ago when the buffaloes were dying these flies used to bite and infect their cattle.

Tabanus fuscomarginatus (Ricardo).

Distribution in Uganda.—Many of these tabanids appeared at Kasala Stream at the foot of Mpumu Hill, and at a forested stream $2\frac{1}{2}$ miles N.N.E. of Mpumu, in the month of April, 1910. These were the only specimens collected by the Commission.

Tabanus albipalpus (Walker).

Distribution in Uganda.—Four specimens were obtained; three by Captain A. E. Hamerton in May from Wakoli's, Usoga ($0^{\circ} 30' \text{ N.}$, $33^{\circ} 45' \text{ E.}$), and one sent by Commander H. Hutchinson on September 24, 1909, from Lake Rubi, Nile.

Description of the Fly.—The fly is small and of a prevailing brown colour, with two greyish stripes down the dorsal surface of the abdomen.

Bionomics.—Nothing is known as to its habits.

Tabanus sp. nov. near *T. insignis* (Loew).

Geographical Distribution.—Up to the present time met with only in Uganda.

Distribution in Uganda.—This fly was obtained from only three places, one of which was in Nsonga, Chagwe, Uganda ($0^{\circ} 15' \text{ N.}$, $32^{\circ} 45' \text{ E.}$), the second near the Massissi River, in Toro ($0^{\circ} 40' \text{ N.}$, $30^{\circ} 40' \text{ E.}$), and the third at Kasala Stream, Mpumu, Uganda. The specimens from the first and last places were obtained by the Commission, and the second by J. H. Leakey, Esq.

Description of the Fly.—Austen says that this fly “belongs to a group of medium-sized or small species, in which the dark-brown or clove-brown body bears sharply defined whitish markings.”

Bionomics.—Its habits are much those of the other members of this genus.

Tabanus irroratus (Surcouf).

Distribution in Uganda.—Two specimens only of this tabanid were found by the Commission on April 28 and July 4, 1910, both at the Kasala Stream (ford at foot of Mpumu Hill), Uganda.

GENUS: *HÆMATOPOTA*.*Hæmatopota vittata* (Loew).Syn.: *Hæmatopota pulchrithorax* (Austen).

(Plate V., "Second Report of the Wellcome Research Laboratories," Khartoum, 1906.)

Distribution in Uganda.—This is one of the commonest and most widely distributed *Hæmatopota* found in Uganda. In 1903 it was sent in from 106 different localities from all parts of the Protectorate. The following table gives the localities from which it was received in 1909-10:—

Table 88.—*Hæmatopota vittata*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° /	° /	
Mar. 8	Fort Portal	—	Toro...	0 40 N.	30 15 E.	Dr. A. Bond.
May 7	Mutanda	Wakoli's	Usoga..	0 30 N.	34 40 E.	Capt. A. E. Hamerton.
June 28	Namukekera.	Bulemezi	Uganda	0 45 N.	32 30 E.	L. C. Lea-Wilson.
July 7	Kiangi ...	Buddu ...	"	0 20 S.	31 8 E.	D. L. Baines
" 26	Mulanda...	Budama..	—	0 37 N.	34 0 E.	P. W. Perryman.
" 26	Peta ...	"	—	0 43 N.	33 58 E.	"
" 26	—	"	—	0 43 N.	33 58 E.	"
Aug. 12	Bulangi ...	Wakoli's	Usogo..	0 30 N.	33 50 E.	E. G. Morris.
" 15	Kampala..	Kiadondo	Uganda	0 20 N.	32 35 E.	Director of Transport.
" 18	Kabula Muliro.	Bulemezi	"	0 40 N.	32 18 E.	Capt. F. P. Mackie.
" 22	Kampala	Kiadondo	"	0 20 N.	32 35 E.	Director of Transport.
Sept. 25	"	"	"	"	"	" "
" 26	"	"	"	"	"	" "
" 27	"	"	"	"	"	" "
" 30	"	"	"	"	"	" "
" 30	"	"	"	"	"	" "
Oct. 15	"	"	"	"	"	" "
" 20	"	"	"	"	"	" "
" 29	"	"	"	"	"	" "
1910.						
April 6	Mpumu ...	Chagwe...	"	0 15 N.	32 50 E.	Commission.
May 18	" ...	" ...	"	"	"	"

Description of the Fly.—It is of a large size for this genus of flies. Its wings are beautifully ornate, with marked ocelli near the tips.

Bionomics.—Attacks man and the domestic animals.

Hæmatopota ugandæ (Ricardo).

(Plate XII., 93, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—Has not, up to the time of publication of Mr. Austen's book, been found outside Uganda.

Table 89.—*Hæmatopota ugandæ*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° /	° /	
May 24	Kasai ...	Chagwe...	Uganda	0 25 N.	32 50 E.	P. W. Cooper.
June 5	Mpumu	"	"	0 15 N.	32 50 E.	Commission.
" 10	"	"	"	0 15 N.	32 50 E.	Capt. F. P. Mackie.
" 13	Sezebwa	"	"	0 18 N.	32 55 E.	"
" 28	Namukera.	Bulemezi	"	0 45 N.	32 30 E.	L. C. Lea-Wilson.
Aug. 10	Kampala	Kiadondo	"	0 20 N.	32 35 E.	Director of Transport.
" 15	"	"	"	"	"	" "
" 31	"	"	"	"	"	" "
Oct. 15	"	"	"	"	"	" "
" 20	"	"	"	"	"	" "
" 29	"	"	"	"	"	" "
1910.						
Mar. 25	Mpumu	Chagwe...	"	0 15 N.	32 50 E.	Commission.

Description of the Fly.—Of full size and uniform brown colour, with well-marked wing ocelli.

Bionomics.—Attacks human beings readily.

Hæmatopota brucei (Austen).

Geographical Distribution.—This species has only been found in Unyoro, Uganda. It was forwarded to Mr. Austen by the Commission in 1903.

Description of the Fly.—This is the largest *Hæmatopota* that has been found in Uganda. The body is black. The posterior extremity of the thorax is boldly marked in white, from which three points of white project forward. The abdomen is crossed by five narrow bands of stone colour. The marking on the wings is diffuse and of a brown colour.

Hæmatopota inornata (Austen).

Distribution in Uganda.—Three specimens were received from Mr. Buckland, Toro, who captured them in the neighbourhood of Butindi (0° 40' N., 30° 15' E.).

Description of the Fly.—This is a large-sized *Hæmatopota*, whose wings lack the intricate pattern which is the rule in other species.

Hæmatopota mactans (Austen).

(Plate XII., 90, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—From Mozambique to Somaliland and Abyssinia.

Distribution in Uganda.—Some specimens were received from P. W. Perryman, Esq., from Budama (0° 37' N., 34° 1' E.).

Description of the Fly.—This is a medium-sized fly, of a general brownish colour, with marked transverse bars of a lighter colour on the dorsal surface of the abdomen. Wings delicately ornamented.

Bionomics.—It was found in several cattle kraals.

Hæmatopota, sp. nov.

Distribution in Uganda.—One specimen of this fly was caught by J. H. Leahey, Esq., on the Mississi River, in Toro (0° 40' N., 30° 40' E.).

Description.—It is rather a small fly, with wing ornamentation of the usual type.

Hæmatopota decora (Walker).

(Plate XII., 89, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—According to Austen, this is a widely distributed species, being found from Cape Colony to the Sudan.

Distribution in Uganda.—This species was sent to the Commission in 1903 from Usoga.

Table 90.—*Hæmatopota decora*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.						
Feb. 1	Muhina...	Sebondo's	Usoga	1 0 N.	33 40 E.	?
" 1	Mbale ...	"	"	1 0 N.	34 0 E.	?
" 1	Waguma	"	"	0 58 N.	33 30 E.	?
" 1	Kasaja ...	"	"	1 0 N.	34 1 E.	?
" 1	Kaja ...	"	"	1 0 N.	34 18 E.	?
" 1	Gandinera	"	"	0 57 N.	34 0 E.	?
" 1	Mubango	"	"	0 58 N.	34 05 E.	?
Mar. 15	Mabira ...	Chagwe...	Uganda	0 35 N.	33 0 E.	E. Brown.
" 21	Jinja ...	—	Usoga	0 30 N.	33 15 E.	Capt. H. M. Tufnell.
Oct. 14	" ...	—	"	0 30 N.	33 15 E.	Capt. G. Lane.

Description of the Fly.—The body of this fly is dark in colour with pale bars running transversely over the dorsal aspect of the abdomen. The wings are beautifully ornate.

Description of the Fly.—A broad, stumpy fly, the abdomen marked by transverse lines of pale buff and two longitudinal rows of large buff-coloured spots.

Habits.—Is said to attack cattle and man viciously.

Hæmatopota tenuis (Austen).

Distribution in Uganda.—Specimens were taken by Captain A. E. Hamerton, R.A.M.C., along the road from Mumia's in North Kavirondo ($0^{\circ} 20' N.$, $34^{\circ} 30' E.$) to Wakoli's in Usoga ($0^{\circ} 30' N.$, $30^{\circ} 45' E.$), where they were very numerous, especially in the forest country.

Description of the Fly.—The fly is described as being small, dusky-grey in colour, with very spotted wings.

Hæmatopota brunnescens (Ricardo).

Distribution in Uganda.—

Table 94.—*Hæmatopota brunnescens*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° ' "	° ' "	
Feb. 17	Namukekera.	Bulemezi	Uganda	0 45 N.	32 30 E.	Commission.
" 27	Mabira ...	Chagwe	"	0 35 N.	33 0 E.	E. Brown.
" 18	Fort Portal.	—	Toro...	0 40 N.	30 15 E.	Dr. A. C. Rendle.
Apr. 23	Mabira ...	Chagwe	Uganda	0 35 N.	33 0 E.	E. Brown.
May —	Mumia's	Kavirondo	—	0 20 N.	34 25 E.	Capt. A. E. Hamerton.
" 7	Hoima ...	Bugoma	Unyoro	1 30 N.	31 30 E.	T. Grant.
" 9	Busesa ...	Wakoli's	Usoga	0 35 N.	33 35 E.	Capt. A. E. Hamerton.
June 18	Lake George.	—	Toro...	0 15 S.	30 0 E.	Dr. A. Bond.
July 1	Mississi...	—	" ...	0 50 N.	30 45 E.	"
" 1	Kitioka...	—	" ...	"	"	"
" 1	Khagora	—	" ...	"	"	"
" 26	Mulanda	Budama	—	0 37 N.	34 0 E.	P. W. Perryman.
" 26	Peta ...	" ...	—	0 43 N.	33 58 E.	"
Aug. 18	Kabula Muliro.	Bulemezi	Uganda	0 40 N.	32 18 E.	Capt. F. P. MacKie.
Oct. 14	Kampala	Kiadondo	"	0 20 N.	32 35 E.	Director of Transport.
" 29	"	"	"	"	"	"
1910.						
Apr. 28	Mpumia.	Chagwe	"	0 15 N.	32 50 E.	Commission.

Description of the Fly.—A medium-sized fly with transverse light-green stripes on abdomen and two rows of green spots, arranged longitudinally. Eyes like black and green watered-silk.

Bionomics.—This fly readily bites man and domestic animals. It has a quick flight and settles suddenly like a tsetse fly, which at first sight it resembles.

Hæmatopota nigrescens (Ricardo).

Distribution in Uganda.—This fly, which resembles *Hæmatopota hirta* (for which it was first mistaken), was caught in a few places in Uganda.

Table 95.—*Hæmatopota nigrescens*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909. —	Mpumu ...	Chagwe...	Uganda	0° 15' N.	32° 50' E.	Commission.
Jan. 1	Mukyanju	Apolo's...	"	1 10 N.	32 40 E.	Sir Apolo Kagwa.
Mar. 28	Kezalizi ...	Mruli ...	Unyoro	1 40 N.	32 10 E.	F. F. Bloedow.
May 9	Busesa ...	Wakoli's	Usoga	0 35 N.	33 35 E.	Capt. A. E. Hamerton.
July 14	Nkomya	Singo ...	Uganda	0 20 N.	32 0 E.	Sir Apolo Kagwa.
" 14	Katiko ...	Mruli ...	"	0 20 N.	32 0 E.	"
" 26	Bunyali ...	Budama...	"	0 45 N.	33 44 E.	P. W. "Perryman."
Aug. 4	Kampala	Kiadondo	Uganda	0 20 N.	32 35 E.	Director of Transport.
" 7	Gondo- koro	—	Nile ...	0 50 N.	31 40 E.	Commission.
" 8	Kibanga...	Chagwe...	Uganda	0 10 N.	32 40 E.	"
" 15	Kampala	Kiadondo	"	0 20 N.	32 35 E.	Director of Transport.
" 31	"	Singo ...	"	0 20 N.	32 35 E.	"
Oct. 1	Gomba ...	Singo ...	"	0 23 N.	32 40 E.	Sir Apolo Kagwa.
" 14	Kiringia...	Jinja ...	Usoga	0 30 N.	33 15 E.	Captain G. Lane.

Description of the Fly.—Besides being rather more sombre it has nothing to render it easily distinguishable from many others of the genus.

Bionomics.—It is a voracious blood-sucker.

Hæmatopota denshamii (Austen).

(Plate XI., 85, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—This fly has only been found in the Nile Province of Uganda and the Lower Sudan.

Distribution in Uganda.—Besides the places in which it was originally caught by the late Dr. Densham, it was found by P. W. Perryman, Esq., in Budama, Bukedi (0° 45' N., 34° 50' E.).

Description of the Fly.—Austen includes this species and two other Uganda species, viz., *Hæmatopota similis* (Ricardo) and *Hæmatopota unicolor* (Ricardo), as being closely allied members of the same group. "In the species belonging to this group the stripes on the dorsum of the thorax are very conspicuous, whilst on the wings the tips and hind borders are much darker than the rest of the surface." (Austen.)

Hæmatopota similis (Ricardo).

Distribution in Uganda.—This species is probably scattered all over Uganda, as it has been sent from most places where fly-catching operations were carried out. The following table gives the particulars of these flies sent:—

Table 96.—*Hæmatopota similis*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1909.							
May 7	Hoima ...	Bugoma ...	Unyoro	0 30 N.	31 30 E.	T. Grant.	Very numerous.
" 7	Mumia's...	—	N. Kavir-ondo.	0 20 N.	34 25 E.	Capt. A. E. Hamerton.	
" 7	Wakoli's...	—	Usoga ..	0 30 N.	34 40 E.	"	
" 7	Busiri ...	—	"	0 25 N.	33 55 E.	"	
" 24	Ntenjeru ..	Bugerere	Uganda	1 0 N.	32 56 E.	P. W. Cooper.	
" 24	Bale ...	"	"	1 0 N.	32 56 E.	"	
" 29	Sesse ...	Lake	"	0 10 S.	32 10 E.	Capt. A. D. Fraser.	
		Victoria.					
July 1	Mississi river.	—	Toro ...	0 50 N.	30 45 E.	Dr. A. Bond.	
" 1	Kagora ...	—	" ...	0 08 N.	31 2 E.	"	
" 1	Mwarra ...	—	" ...	0 08 N.	31 20 E.	"	
Aug. 1	Mpumu ...	Chagwe ..	Uganda	0 15 N.	32 50 E.	Capt. H. R. Bateman.	
" 6	Mississi river.	—	Toro ...	0 40 N.	31 0 E.	J. H. Leakey.	
" 22	Kampala	Kiadondo	Uganda	0 20 N.	32 35 E.	Director of Transport.	
" 25	"	"	"	0 20 N.	32 35 E.	"	
" 31	"	"	"	0 20 N.	32 35 E.	"	
Oct. 1	Gomba ...	Singo ...	"	0 23 N.	32 40 E.	Sir Apolo Kagwa.	
1910.							
May 1	Mpumu ...	Chagwe ..	"	0 15 N.	32 50 E.	Commission.	

Description of the Fly.—This resembles *Hæmatopota denshamii*, and is of a dusky-brown with dark edges to wings. The eyes are of a beautiful shot-silk texture, iridescent green, with five black bands.

Bionomics.—Attacks man very readily and bites viciously.

Hæmatopota unicolor (Ricardo).

Distribution in Uganda.—The following table shows the general distribution:—

Table 97.—*Hæmatopota unicolor*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1909. Feb. 17	Namukekera.	Bulemezi	Uganda	0° 45' N.	32° 30' E.	Capt. H. R. Bateman and F. P. Mackie.	
Apr. 15	"	"	"	0° 45' N.	32° 30' E.	"	Very numerous.
" 16	"	"	"	0° 45' N.	32° 30' E.	L. C. Lea-Wilson.	
May 7	Mumia's...	—	N. Kavirondo.	0° 20' N.	34° 25' E.	Capt. A. E. Hamerton.	Very numerous.
" 7	Wakoli's	—	"	0° 30' N.	34° 40' E.	"	"
" 25	Masaka ...	Buddu ...	Uganda	0° 20' S.	31° 50' E.	D. L. Baines.	
" 29	Sesse islands.	Lake Victoria.	"	0° 10' S.	32° 10' E.	Capt. A. D. Fraser.	
July 22	Kabula Muliro.	Bulemezi	"	0° 40' N.	32° 18' E.	L. C. Lea-Wilson.	
" 31	Namukekera.	"	"	0° 45' N.	32° 30' E.	"	
Aug. 6	"	"	"	0° 45' N.	32° 30' E.	"	
" 7	"	"	"	0° 45' N.	32° 30' E.	"	
" 7	Kiango ...	Buddu ...	"	0° 20' S.	31° 8' E.	D. L. Baines.	
" 12	Bulangi ...	Wakoli's	Usoga...	0° 30' N.	33° 50' E.	E. G. Morris.	
" 18	Kabula Muliro.	Bulemezi	Uganda	0° 40' N.	32° 18' E.	Capt. F. P. Mackie	Numerous.
" 18	"	"	"	0° 40' N.	32° 18' E.	"	Ford.
—	Namukekera.	"	"	0° 45' N.	32° 30' E.	"	Game track.
" 20	Busoa ...	"	"	0° 41' N.	32° 22' E.	Capt. A. E. Hamerton.	
Oct. 1	Wamala ...	Singo ...	"	0° 25' N.	33° 15' E.	Sir Apolo Kagwa.	
" 14	Mpumu ...	Chagwe ...	"	0° 15' N.	32° 50' E.	Commission ...	On cattle.
" 14	Jinja ...	—	Usoga ..	0° 30' N.	33° 15' E.	E. Hutchins ...	
Nov. 2	Lukayu ...	Mbarara...	Ankole	0° 35' S.	30° 40' E.	Dr. A. C. Rendle.	"

Description of the Fly.—This is a buff, smoky-coloured fly, which has a maroon-coloured *Tabanus*-like head without ocelli. The eyes are separated by a narrow sulcus only. The wing markings resemble those of other *Hæmatopota*. Austen includes this species in a group with *Hæmatopota similis*, *Hæmatopota denshamii*, and some others.

Bionomics.—This fly is a very voracious blood-sucker, and attacks cattle with avidity. A thin trickle of blood marks the site of the puncture and usually attracts large numbers of *Stomoxys*, who are thus able to get a blood feed without using their own piercing apparatus. This *Hæmatopota* attacks man readily in default of cattle, and sportsmen are frequently bitten when walking through the fly areas in search of game.

GENUS: CHRYSOPS.

Chrysops brucei (Austen).

(Plate III., 17, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—This fly has not been recorded as occurring outside the limits of Uganda and the Anglo-Egyptian Sudan.

Distribution in Uganda.—

Table 98.—*Chrysops brucei*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1908.						
Dec. 8	Viatente...	—	—	° —	° —	Ham Mukasa, Sekibobo.
" 8	Mpumu ...	Chagwe...	Uganda	0 15 N.	32 50 E.	Commission.
1909.						
Mar. 28	Kezalizi ...	Mruli ...	Unyoro	1 40 N.	32 10 E.	F. F. Bloedow.
July 14	Wamala...	Singo ...	Uganda	0 25 N.	33 15 E.	Sir Apolo Kagwa.
" 14	Katiko ...	"	"	0 20 N.	32 0 E.	"
Aug. 4	Kampala	Kiadondo	"	0 20 N.	32 35 E.	Director of " Transport.
" 6	"	"	"	"	"	" "
" 15	"	"	"	"	"	" "
" 19	"	"	"	"	"	" "
" 25	"	"	"	"	"	" "
" 31	"	"	"	"	"	" "
Sept. 4	Jinja ...	—	Usoga	0 30 N.	33 15 E.	Dr. H. B. Owen.
" 7	Kampala	Kiadondo	Uganda	0 20 N.	32 35 E.	Director of " Transport
" 9	"	"	"	"	"	" "
" 14	"	"	"	"	"	" "
Oct. 14	Jinja ...	—	Usoga	0 30 N.	33 15 E.	Capt. G. Lahe.
" 15	Kampala	Kiadondo	Uganda	0 20 N.	32 35 E.	Director of " Transport.
" 29	"	"	"	"	"	" "

Description of the Fly.—A medium-sized species of greenish colour. The wings are nearly transparent except for a dark-olive patch about the middle of the upper margin.

Bionomics.—They are said to be determined blood-suckers, attacking men and animals.

Chrysops distinctipennis (Austen).

(Plate II., 12, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—According to Austen, this species has a wide distribution in tropical Africa, being met with in many parts of West Africa as well as in Abyssinia, Uganda, and the Congo.

Distribution in Uganda.—Table 99.—*Chrysops distinctipennis*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° /	° /	
Feb. 1	Mahina	Sebondo's	Usoga	1 0 N.	33 40 E.	Prov. Commis- sioner of Jinja.
" 1	Waguma	"	"	0 58 N.	33 30 E.	"
" 1	Kaja ...	"	"	1 0 N.	"	"
" 17	Namukekera	Bulemezi	Uganda	0 45 N.	32 30 E.	Capt. H. R. Bateman and F. P. Mackie.
July 7	"	"	"	"	"	L. C. Lea-Wilson.
" 26	Mulanda	Budama	"	0 37 N.	34 0 E.	P. W. Prettyman.
" 31	Namukekera.	Bulemezi	Uganda	0 45 N.	32 30 E.	L. C. Lea-Wilson
Aug. 6	"	"	"	"	"	"
" 12	Kumi ...	"	Bukedi	1 30 N.	33 20 E.	E. G. Morris.
" 18	Kabula	Bulemezi	Uganda	0 40 N.	32 18 E.	Capt. F. P. Mackie.
" 18	Muli.	"	"	"	"	"
" 18	Various places near above.	" ...	"	"	"	"
Sept. 7	Kampala	Kiadondo	"	0 20 N.	32 35 E.	Director of Transport.
Oct. 20	"	"	"	"	"	"
" 29	"	"	"	"	"	"

Description of the Fly.—This fly is about the same size, but of brighter colour, than the last-mentioned species. The wings are similarly marked with a red-brown patch, the remainder being clear.

Bionomics.—Little is known as to its habits.

Chrysops funebris (Austen).

(Plate II., 9, Austen's "African Blood-sucking Flies.")

Geographical Distribution.—According to Austen, this fly, up to the present, has only been reported from Uganda.

Distribution in Uganda.—In 1910 this species was frequently found at the Kasala Stream at the foot of Mpumu, Chagwe, Uganda (0° 15' N., 32° 50' E.).

Chrysops centurionis (Austen).

"Bulletin of Entomological Research," Vol. II., p. 164, Fig. 2 (1911).

Geographical Distribution.—This species has at present not been found outside Uganda.

Distribution in Uganda.—In 1910 this species was found, on a few occasions, near the same stream, at Mpumu, Chagwe, Uganda. Captain A. D. Fraser states that they were common at Buwanuka, in Singo.

GENUS: RHINOMYZA.

Rhinomyza perpulcra (Austen).

This handsome species was only found at the Kasala Stream, near Mpumu. They were rather rare, and were usually caught settling on the stones in the stream during the hottest time of the day. They were caught where the wood was thick; and the natives said they came down from the trees.

FAMILY: MUSCIDÆ.

GENUS: STOMOXYS.

Stomoxys nigra (Macquart).

Stomoxys calcitrans (Linnæus).

(Plate XIII., 101 and 102, Austen's "African Blood-sucking Flies.")

Native name: "Kawawa."

These are the small biting flies which are so numerous in the vicinity of cattle kraals, and which closely resemble the common house-fly. These are the two common species in Uganda, but as in many cases no attempt was made to distinguish between them, the two will here be considered together.

Geographical Distribution.—This genus of flies is probably of universal distribution in Africa.

Distribution in Uganda.—These flies are ubiquitous. They were sent in, in great numbers, from all parts of the country.

Description of the Fly.—These flies closely resemble ordinary house-flies in size, colouring, and general appearance. On close inspection, however, they will be seen to bear a short, sharp, needle-like proboscis, which projects forwards, and which at once distinguishes them from the common house-flies.

Bionomics.—They attack man, cattle, and the other domestic animals, driving their short proboscis deeply into the skin, and quickly filling themselves with blood. Their bite feels like a needle prick, but is not generally followed by any after-effect. They lay their eggs in dung and other fermenting vegetable matter, and the larva or maggot passes this stage of its existence in this material.

GENUS: GLOSSINA.

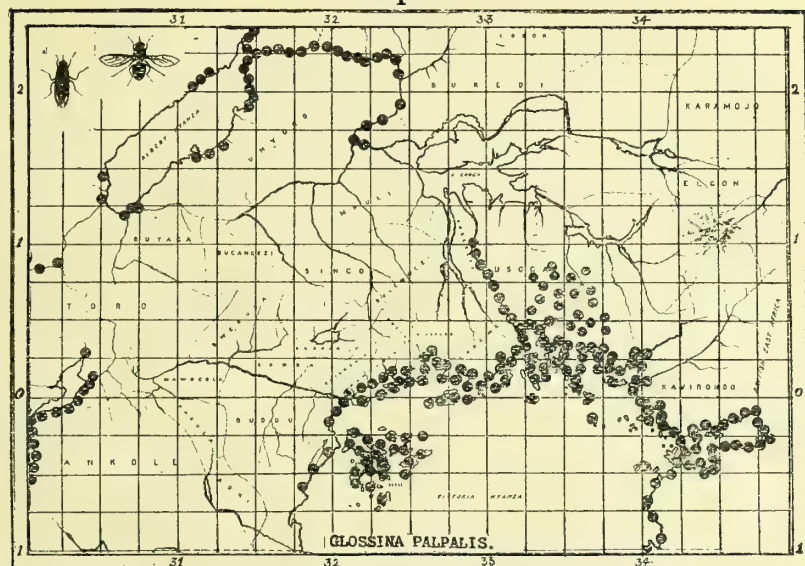
Glossina palpalis (Rob.-Desv.).

(Plate I., Austen's "Handbook of the Tsetse-Flies.")

Native name: "Kivu," singular; "Bivu," plural.

Distribution in Uganda.—Besides the wide areas of the Lake coast line where this tsetse fly swarms, the following inland places were reported as being fly-infected:—

Map. 7.

Table 100.—*Glossina palpalis*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.						
Feb. 22	Kachwamba	Kitakwenda	Ankole	0° 0'	30° 25' E.	Dr. A. C. Rendle.
Mar. 3	Ngussi river	Bugoma...	Unyoro	1° 15' N.	30° 45' E.	T. Grant.
Apr. 11	Wasa river	—	Toro ...	0° 30' N.	30° 20' E.	Dr. A. Bond.
" 16	Ngussi river	Bugoma...	Unyoro	1° 15' N.	30° 45' E.	T. Grant.
May 10	Sio river...	—	Usoga	0° 25' N.	34° 10' E.	Capt. A. E. Hamerton.
" 27	Kokoi river	W. of Lake Albert	Unyoro	1° 20' N.	30° 30' E.	T. Grant.
June 16	Mahigi ...	—	"	2° 15' N.	31° 20' E.	Capt. H. M. Tufnell.
" 18	Massissi river	—	Toro ...	0° 40' N.	30° 40' E.	Dr. A. Bond.
July 2	Lake Albert	—	Unyoro	2° 0' N.	31° 25' E.	Dr. J. H. Goodliffe
" 23	Wasa river	—	Toro ...	0° 30' N.	30° 20' E.	Dr. A. Bond.
" 23	Kyebonekire	—	"	"	"	"
Aug. 4	Kampala	Kiadondo	Uganda	0° 20' N.	32° 35' E.	Director of Transport.
Sept. 3	Bussu ...	—	Usoga	0° 30' N.	33° 25' E.	Dr. H. Bayon.
" 24	Lake Rubi	Nile ...	—	—	—	Commander H. Hutchinson.

Description of Fly.—The following description is given by Mr. Austen ("Handbook of the Tsetse-Flies.") :—

"Abdomen clove-brown or blackish-brown; thorax usually paler, with dark brown markings on a greyish ground; abdomen generally with at least an indication of a pale or slate-grey longitudinal median stripe, with pale lateral triangular markings, and usually the hind margins of the segments narrowly pale. Femora in typical race more or less mouse-grey, greyish-brown, or dark slate-coloured; tibiæ, extreme tips of femora, and first three joints of front and middle tarsi buff or ochraceous-buff,

The presence of this fly has only been reported in a few parts of Uganda, but, nevertheless, a map is given, even if it only serves to show how little is known of the distribution of this very important tsetse fly, and to stimulate other observers to fill up the lacunæ in our knowledge. Commissioner T. Grant, of Hoima, found some in the Bugoma forest district, in Unyoro ($1^{\circ} 20' N.$, $31^{\circ} 0' E.$) It has been found in considerable numbers by Assistant Commissioner E. B. Haddon and other travellers up the Nile, especially on the road between Nimule and Gondokoro.

Description of the Fly.—Austen summarises the description of the fly as follows:—"Dorsum of thorax light grey, olivaceous-grey, or smoke-grey in the male, drab-grey in the female, the thoracic markings in both sexes incompletely developed, and reduced to brownish or mouse-grey longitudinal streaks; dorsum of abdomen buff to ochraceous-buff (*in pinned specimens sometimes mouse-grey or olivaceous owing to post-mortem changes*), with a larger or smaller clove-brown blotch (sometimes indistinct or almost wanting) near each basal angle of the second segment, and the third to the sixth segments inclusive each with a very conspicuous clove-brown transverse band, interrupted in the median line, not reaching the lateral margins, and not extending beyond the basal three-fourths of the segment, if so far; legs buff, last two joints of hind tarsi clove-brown or black, last two joints of front tarsi and penultimate joint of middle tarsi conspicuously tipped with clove-brown or dark brown, last joint of middle tarsi entirely dark brown above in typical race, otherwise distal half or third of last joint of middle tarsi alone dark brown or clove-brown, remainder of joint merely brownish or even entirely pale."

Bionomics.—Like the foregoing species, this fly produces a single living larva at a birth, which creeps away to enter at once on its pupal stage, and later to emerge as the perfect insect. The fly is a voracious blood-sucker, and attacks man and other animals savagely. It is responsible for carrying the virus of "tsetse-fly disease" of South African stock.

Glossina pallidipes (Austen).

(Plate VI., Austen's "Handbook of the Tsetse-Flies.")

Geographical Distribution.—This tsetse fly resembles *Glossina morsitans* very closely, and in many returns they are included under one name. The distribution of this species may be taken as similar to that of *Glossina morsitans*.

Distribution in Uganda.—Specimens of true *pallidipes* were forwarded from three districts. Some were sent by Dr. A. Bond, and were caught in the Kijura district, two hours from Lake Albert, and near the Massissi River. Others were sent by Dr. J. H. Goodliffe, from Tonio, a village situated on the eastern shores of Lake Albert ($1^{\circ} 35' N.$, $31^{\circ} 10' E.$). H. W. Taggart, Esq., also sent some specimens from the neighbourhood of Masindi, in Unyoro ($1^{\circ} 40' N.$, $32^{\circ} 0' E.$).

Description of the Fly.—Austen gives the following description:—"Medium-sized or rather large species, closely resembling the West African *Glossina longipalpis* (Wied.), but distinguishable owing to the coloration of the last two joints of the front and middle tarsi. Front in the male very narrow; dorsum of thorax olive-grey, smoke-grey, or drab-grey, with the usual dark brown, longitudinal markings generally much reduced, broken up, and but little conspicuous; abdomen buff, dorsum with dark brown or clove-brown bands; legs buff, last two joints of hind tarsi clove-brown, front and middle tarsi either entirely pale or, at most, last two joints of front tarsi faintly brownish at the tips, and last joint and distal half of penultimate joint of middle tarsi light brown—never so dark as to form a sharp contrast with the remaining joints."

Bionomics.—The habits appear to be similar to those of *Glossina morsitans*.

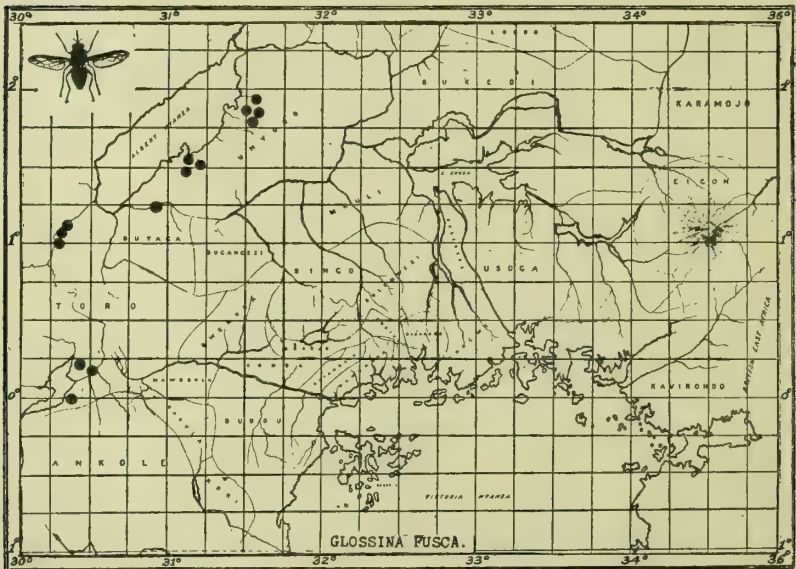
Glossina fusca (Walker).

(Plate VIII., Austen's "Handbook of the Tsetse-Flies.")

Geographical Distribution.—It has been recorded from the following regions of equatorial Africa, generally occurring in small patches or belts: Uganda, the Belgian and French Congo, the Cameroons, the Niger Basin, and many places in West Africa.

Distribution in Uganda.—

Map 9.



No specimens of this fly were received by the present Commission at Mpumu. The localities marked on the map are those given by Dr. A. G. Bagshawe, the Director of the Sleeping Sickness

Bureau, and were the result of his own observations when travelling in Uganda.

Description of the Fly.—"Large, dark brown, dark-winged species, with long and slender palpi, and without distinct dark bands on the abdomen, which is lighter at the base; in general appearance very closely resembling *Glossina nigrofusca* (Newst.), but distinguishable in both sexes by the third joint of the antennæ not being clothed with long and fine pale hairs, forming a conspicuous fringe down the anterior and posterior edges." Austen.

Bionomics.—The breeding and life habits of this species have not been worked out. It is a great pest to cattle, and is capable of giving a very severe bite; it attacks man also.

FAMILY: SIMULIIDÆ.

GENUS: SIMULIUM.

Simulium damnosum (Theobald).

(Plate I., 6, Austen's "African Blood-sucking Flies.")

Native name: "Mbwa" fly. Synonym: Sand-fly.

Geographical Distribution.—There is little doubt but that this species is distributed throughout equatorial Africa.

Distribution in Uganda.—The presence of this fly has been noticed previously by Dr. Cuthbert Christy and others, and in some parts are a terrible pest. Specimens were sent to the present Commission as follows:—

Table 101.—*Simulium damnosum*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° ' "	° ' "	
Jan. 10	Kibanga	Chagwe	Uganda	0 15 N.	32 50 E.	Capt. A. E. Hamerton.
" 10	Mabira	"	"	0 35 N.	33 0 E.	Col. Sir David Bruce.
Feb. 1	Muhina	Sebondi's	Usoga	0 54 N.	33 58 E.	Commissioner, Jinja.
" 1	Wajalulu	"	"	0 55 N.	33 28 E.	"
Mar. 2	Mabira	Chagwe	Uganda	0 35 N.	33 0 E.	E. Brown.
May 24	Ntenjeru	Bugerere	"	1 0 N.	32 56 E.	P. W. Cooper.
" 24	Sezebwa River.	Chagwe	"	—	—	"
July 22	Mpumu	"	"	0 15 N.	32 50 E.	Commission.

Description of the Fly.—They are minute flies of black or greyish-black colour, and their bodies are covered with delicate hairs. Their wings, which are large and wide, are of a beautiful iridescent colour.

Bionomics.—The males do not suck blood. The females bite severely, and the bite produces much irritation. They bite early in the morning and through the day, and as they are small enough to pass through the meshes of mosquito-netting, they are often a perfect scourge to travellers.

FAMILY: CHIRONOMIDÆ.

GENUS: CULICOIDES.

Culicoides brucei (Austen).

Geographical Distribution.—Has only been found on the Myanja River, Uganda. Specimens were sent to Mr. Austen by the Commission in 1903.

Description of the Fly.—Very minute flies of a uniform brownish colour, with spotted wings.

Culicoides milnei (Austen).

Geographical Distribution.—Has only been found at Nairobi, and the Athi Plains, British East Africa, and Wakoli's, Busoga, Uganda.

Description of the Fly.—Very minute flies, rather larger than *Culicoides brucei*, of a uniform brownish colour, with spotted wings.

Bionomics.—The females bite viciously, especially in the early mornings.

FAMILY: HIPPOBOSCIDÆ.

GENUS: ECHESTYPUS.

Echestypus paradoxus (Newstead).

Only one species of this family was collected, and that off a bush-buck, sent from Singo, May 23, 1910.

44.—DISTRIBUTION OF TICKS IN UGANDA.

Introduction.

The three most important ticks in Uganda are the Variegated, the Brown, and the Blue tick. They are all three of almost universal distribution in Uganda. One curious exception to this is that the Brown tick, the carrier of East Coast fever, is unknown in the Sesse Islands. The result of this was, that the cattle reared on the Islands remained susceptible to this disease, and on their arrival to the mainland died of this disease in large numbers.

The following account of the Uganda ticks is very defective, but it may be useful as a basis for the accumulation of further knowledge.

The species dealt with are *Amblyomma variegatum*, *A. eburneum*, *A. tholloni*, *A. petersi*, *A. sparsum*, *A. splendidum*; *Rhipicephalus appendiculatus*, *R. simus*, *R. capensis*, *R. evertsi*; *Boophilus decoloratus*; *Hæmaphysalis leachi*; *Ornithodoros moubata*, and *O. savignyi*.

FAMILY: IXODIDÆ.

GENUS: AMBLYOMMA.

Amblyomma variegatum (Fabricius).

The Variegated Tick.

Native names: "Enkwa"; "Embalabala."

Geographical Distribution.—It is almost universally distributed throughout tropical Africa, and is found, but less frequently, in South Africa. Its range, however, extends from Sierra Leone to Abyssinia, northwards to the Sudan, and southwards into Cape Colony and Madagascar.

Distribution in Uganda.—Very large numbers of this tick were sent from various collectors in Uganda—indeed, it seemed to be universally distributed. The following table gives the full details.

Table 102.—*Amblyomma variegatum*.

Date.	Locality.	District.	Province.	Lat.*	Long.*	Donor.	Remarks.
1908.							
Dec. 16	Kajingolo	Mitiana ...	Gomba ...	0° 25' N.	32° 12' E.	Sir Apolo Kagwa.	
" 21	Kigejo ...	Budonga ...	Unyoro ...	1° 50' N.	31° 50' E.	"	
" 23	Businja ...	Bulemezi ...	Uganda ...	0° 40' N.	32° 30' E.	"	
" 28	Bugajya ...	Lewankoli	Buddu	Between 0° 0' & 1° 0' S. and 31° 0' E. & 32° 20' E.	}	"	
" 28	Kisanje ...	Kamaliya	"				
" 28	Kasambya	Kichwalu- gingo.	"				
" 28	Biga ..	Bugaga ...	"				
" 28	Kanywa ...	Buyanja ...	"	1° 0' N.	32° 50' E.	"	
" 28	—	Butembala	"				
" 28	—	Bugerere	Chagwe				
1909.							
Jan. 1	—	Mugungula	?	?	?	"	
" 6	—	Buruli ...	Uganda ...	1° 20' N.	32° 20' E.	"	
" 9	Lukokome	Koki ...	"	1° 0' S.	31° 32' E.	"	
" 9	Mabrio ...	"	"	1° 0' S.	31° 32' E.	"	
" 22	Lubembe	Bulemezi	"	0° 35' N.	32° 30' E.	"	
Feb. 3	Namuke- kera.	"	"	0° 45' N.	32° 30' E.	Cpts. Bateman and Mackie.	Off cattle.
" 6	Kalare	"	"	0° 36' N.	32° 40' E.	Sir Apolo Kagwa.	
" 9	Namuke- kera.	"	"	0° 45' N.	32° 30' E.	Cpts. Bateman and Mackie.	Off cobus.
" 9	"	"	"	0° 45' N.	32° 30' E.	"	Off water- buck.
" 12	Entebbe ...	—	"	0° 04' N.	32° 26' E.	Director of Transport.	
" 27	Mubango...	Chagwe ...	"	0° 35' N.	33° 0' E.	E. Brown.	
" 27	Forest ...	"	"	0° 35' N.	32° 52' E.	Sir Apolo Kagwa.	

* The degrees of latitude and longitude are accurate only as regards the district and not necessarily as regards the locality

Table 102.—*Amblyomma variegatum*—continued.

Date.	Locality.	District.	Province.	Lat.*	Long.*	Donor.	Remarks.
1909.							
Mar. 3	Mumianta	Buruli ...	Uganda ...	0 16 N.	32 55 E.	Sir Apolo Kagwa.	
" 4	Sale ...	Gomba ...	"	0 20 N.	32 0 E.	"	
" 4	Makakwa	"	"	0 20 N.	32 0 E.	"	
" 4	Mamba ...	"	"	0 20 N.	32 0 E.	"	
" 10	Namuke- kera.	Bulemezi	"	0 45 N.	32 30 E.	L. C. Lea-Wilson	Off zebra.
" 11	Kimaga ...	Buruli ...	"	1 30 N.	32 30 E.	Sir Apolo Kagwa.	
" 11	Kazwama	"	"	1 20 N.	32 40 E.	"	
" 11	Kiguli ...	"	"	1 24 N.	32 50 E.	"	
" 16	Lukage ...	Bulemezi	"	0 30 N.	32 15 E.	"	
" 16	Limba ...	"	"	0 29 E.	32 18 E.	"	
" 16	Kibuze ...	"	"	0 31 N.	32 16 E.	"	
" 16	Kiguli ...	"	"	0 30 N.	32 20 E.	"	
" 18	Makote river.	Buruli ...	"	1 50 N.	32 50 E.	Capt. H. M. Tufnell.	
" 19	Katonga river.	Gomba ...	"	0 05 N.	31 40 E.	H.H. the Kabaka.	Cattle dying.
" 24	Jinja ...	Usoga ...	"	0 30 N.	33 15 E.	Capt. H. M. Tufnell.	
" 27	Kabugenda	Buruli ...	"	1 30 N.	32 20 E.	"	
April 1	Mpumumu ...	Chagwe ...	"	0 15 N.	32 50 E.	Commission.	
" 1	Kabula	Bulemezi	"	0 40 N.	32 18 E.	Dr. R. van Someran.	
" 1	Muliro	Chagwe ...	"	0 35 N.	33 0 E.	E. Brown.	
" 1	Mabira ...	Ankole ...	Western...	0 32 N.	30 45 E.	Dr. A. C. Rendle.	
" 1	Mbarara ...	"	"	0 30 S.	30 45 E.	"	
" 19	Mbale ...	—	Bukedi ...	1 0 N.	34 0 E.	Dr. J. A. Taylor.	
" 19	—	Bulemezi	Uganda ...	0 35 N.	31 15 E.	Dr. J. M. Collins.	On wart- hog.
" 23	Hoima ...	—	Unyoro ...	1 25 N.	31 25 E.	L. C. Lea-Wilson.	
" 23	Mabira ...	Chagwe ...	Uganda ...	0 35 N.	33 0 E.	E. Brown.	
" 26	Masero ...	Kavirondo	Eastern ...	1 10 N.	34 10 E.	Capt. A. E. Hamerton.	
" 30	Madu ...	Gomba ...	Uganda ...	0 08 N.	31 40 E.	Sir Apolo Kagwa.	
" 30	Kalwanga	"	"	0 08 N.	31 40 E.	"	
" 30	Lusongoda	"	"	0 10 N.	31 38 E.	"	
May 12	Mululi ...	Unyoro ...	"	1 40 N.	32 8 E.	Dr. R. van Someran.	
" 24	Semukutu	Buruli ...	—	1 20 N.	32 20 E.	P. W. Cooper.	
" 24	Nasongola	—	—	1 20 N.	32 20 E.	"	
June 9	Masaka ...	Buddu ...	Uganda ...	0 20 S.	31 50 E.	Sir David Bruce.	
" 23	Mpumumu ...	Chagwe ...	"	0 15 N.	32 50 E.	Commission ...	On a goat.
" 26	Bussu ...	Usoga ...	Eastern ...	0 30 N.	33 30 E.	Dr. H. Bayon.	
July 1	—	Toro ...	—	0 30 N.	30 15 E.	J. D. Buckland ...	On an elephant.
" 3	Bululu ...	Bukedi ...	Central ...	1 40 N.	33 20 E.	G. P. Jervoise ...	
" 13	—	Ankole ...	Western	0 30 S.	30 30 E.	Dr. C. Wiggins ...	On harte- beeste.
" 13	—	"	"	0 30 S.	30 25 E.	"	On topi.
" 20	Kigule ...	Buruli ...	Uganda ...	1 23 N.	32 30 E.	Sir Apolo Kagwa.	
" 20	Dogola ...	"	"	1 25 N.	32 21 E.	"	
" 23	Sesse ...	Lake Victoria.	—	0 30 S.	32 10 E.	Dr. C. H. Marshall.	
" 23	—	—	Uganda ...	0 30 S.	32 10 E.	Col. L. C. E. Wyndham.	
" 23	Lugogo ...	—	Bukedi ...	1 6 N.	32 50 E.	Sir Apolo Kagwa.	
" 23	Junda ...	—	"	1 5 N.	32 45 E.	"	
" 23	Kanabu- lema.	Masaka ...	Uganda ...	0 22 S.	31 38 E.	D. L. Baines.	
" 23	Kiangoo	"	"	0 20 S.	31 36 E.	"	
" 26	Nagongera	Budama ...	—	0 40 N.	33 0 E.	P. W. Perryman.	
" 26	Kakingeru	Mbale ...	—	1 0 N.	34 0 E.	"	
" 26	Butelein	Bunyuli ...	—	1 5 N.	34 49 E.	"	
" 31	Kabazi ...	Buruli ...	Bukedi ...	1 22 N.	32 20 E.	Sir Apolo Kagwa.	
" 31	Kampala	Kiadondo	Uganda ...	0 20 N.	32 35 E.	Director of Transport.	
Aug. 6	"	"	"	0 20 N.	32 35 E.	"	

* The degrees of latitude and longitude are accurate only as regards the district and not necessarily as regards the locality.

Table 102.—*Amblyomma variegatum*—continued.

Date.	Locality.	District.	Province.	Lat.*	Long.*	Donor.	Remarks.
1909.							
Aug. 6	Mazigo ...	Masaka ...	Uganda ...	0° 20' S.	31° 40' E.	D. L. Baines ...	Off Sesse cattle.
" 6	Bunyago ...	Buddu ...	"	0° 15' S.	31° 30' E.	"	"
" 6	Masaka ...	"	"	0° 20' S.	31° 50' E.	"	"
" 7	Gondokoro	—	Nile ...	0° 50' N.	31° 40' E.	E. B. Haddon,	
" 12	Kampala...	Kiadondo	Uganda ...	0° 20' N.	32° 35' E.	Director of Transport.	
" 12	Kidongoli	Bukedi ...	Central	Between 1° 0' & 2° 0' N. and 33° 0' & 34° 0' E.		E. G. Morris.	
" 12	Kachumbala.	"	"				
" 12	Soroli ...	"	"				
" 12	Magora ...	"	"				
" 12	Mkongoro	"	"				
" 12	Bugiro ...	"	"				
" 12	Kapiri ...	"	"				
" 12	Malera ...	"	"				
" 12	Mukulu ...	"	"				
" 12	Ngola... ..	"	"				
" 12	Bukedea...	"	"				
" 12	Kobeolvi ...	"	"				
" 13	—	—	Uganda ...	?	?	Rev. E. Millar.	N. shore of Lake Chioga.
" 17	Namusale	"	Central ...	1° 30' N.	33° 20' E.	G. P. Jervoise ...	
" 18	Kampala...	Kiadondo	Uganda ...	0° 20' N.	32° 35' E.	Director of Transport.	
" 20	Buyinge ...	Sebondi's	Usoga ..	0° 55' N.	33° 40' E.	F. M. Isemonger.	Calves died of amakebe.
" 20	—	"	"	0° 56' N.	33° 40' E.	"	
" 20	—	"	"	0° 56' N.	33° 38' E.	"	
" 20	Bugiri ...	"	"	0° 25' N.	33° 40' E.	"	
" 20	Budiopo ...	Bulembo..	"	0° 20' N.	33° 42' E.	"	
Sept. 14	Namirembe	Kiadondo	Uganda ...	0° 18' N.	31° 32' E.	C. J. Phillips ...	
" 14	Ngora ...	—	—	1° 40' N.	34° 10' E.	?	From sheep.
" 29	Mbarara ...	Ankole ...	Western	0° 30' S.	30° 45' E.	Dr. A. C. Rendle.	
Oct. 14	Kerungia	Jinja ...	Usoga ...	0° 25' N.	33° 15' E.	Capt. G. Lane.	
" 18	Mugema ...	Kitala ...	—	—	—	Dr. C. Wiggins.	

* The degrees of latitude and longitude are accurate only as regards the district and not necessarily as regards the locality.

Description.—To give an accurate description of the minute morphological characters which serve to differentiate the species of these parasites one from the other presupposes a considerable degree of entomological knowledge. Such descriptions are considered outside the scope of this paper, which is written chiefly to assist the agriculturalist and stock-breeder to identify the commoner parasites which he may meet with in Uganda. The practice which was adopted in the case of the biting flies will therefore be continued here, namely, that of giving a short description of the most obvious external characters of the tick and a list of the localities where the species was found, with the name of the donor. Those who wish to obtain a fuller and more scientific description of these parasites are referred to one or other of the following books:—

1. A List of the Ticks of South Africa. By C. W. Howard, in the *Farmers' Bulletin*, No. 30, of the Transvaal Department of Agriculture, Pretoria, 1908.
2. Article on Ticks. By R. I. Pocock, F.Z. S., in Clifford Allbutt's "System of Medicine," Macmillan & Co., 1907.

Adult Male.—A medium-sized tick, whose back is beautifully marked with copper and green. The middle of the upper surface is of a copper-red colour, behind paling to primrose-yellow; in front and around this copper shield there is a wide margin of metallic-green. The posterior margin of the upper surface is marked by a series of longitudinal festoons, or serrations, which are green in colour and bordered with yellowish-white. The legs are stout and of reddish-brown colour with white markings.

Adult Female.—Is larger than the male, but of similar shape, and not nearly so handsome. The prevailing colour of the upper surface is deep-brownish green, and in front there is a narrow copper shield limited to the anterior third of the upper surface. The legs are longer and the junctions of the various joints are picked out with white bands. The legs, therefore, looked speckled with red and white.

The Nymph.—Is an inconspicuous-looking creature; very small, but with the general shape of the female tick. The upper surface is reddish-brown and without metallic lustre.

The larva.—Is a minute object, little more than half a millimetre in length. It is possessed of great activity.

Bionomics.—This tick is very common on cattle. The male is active and wanders about in search of the female. The latter when engorged and fecundated reaches an enormous size ("24 mm. long by 15 mm. wide, and almost as thick." Howard). When in this state it is of dirty-brown colour, and forms a very prominent object. It is seen clinging to cattle generally in the region of the anus, or in the folds of the groin, or scrotum. The tick always leaves the host to moult. As will be seen from the above table the parasite feeds on a variety of animals, such as cattle, sheep, goats, various kinds of antelope, zebra, and on elephants, and according to Howard it is found also on the rhinoceros. This tick is very closely related to *Amblyomma hebraeum*, which transmits "heartwater" to sheep and calves. It may be a host of *Piroplasma parvum* (East Coast fever).

Amblyomma eburneum (Gerstaecker).

Geographical Distribution.—Stated to be the same as *Amblyomma hebraeum*, i.e. Cape Colony in the south to Abyssinia and the Congo in the north. Neither *Amblyomma hebraeum* nor this sub-variety is stated to occur in Uganda.

Distribution in Uganda.—Only three specimens were received, and they were found by E. G. Morris, Esq., at a locality called Magora, in Kumi District, in the Bukedi sub-Province (1° 30' N., 33° 30' E.).

Description.—This tick closely resembles, and for practical purposes may be classed with, *Amblyomma hebraeum* (Koch). The male is of "shining sulphur-yellow, with spots and lines of a deep chestnut colour." (Howard.) The festoons are of the same yellow colour. In *Amblyomma eburneum* the dark markings are wider and better marked, and the colour contrast is therefore stronger than in *Amblyomma hebraeum*. The legs in both sexes are reddish-brown and only faintly dotted with white. The female

is less ornate than the male and of a general dark-brown colour. When engorged she reaches an enormous size, and closely resembles the distended female of *Amblyomma variegatum*.

Bionomics.—The allied *Amblyomma hebraeum* transmits "heart-water" in sheep and calves. The life-history of the two is similar if not identical.

Four other species of the genus *Amblyomma* were met with, but none of them are at present known to possess any economic importance.

Amblyomma tholloni.—This species was only found on wild elephants.

Distribution in Uganda.—

Table 103.—*Amblyomma tholloni*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1909.				° /	° /	
Apr. 16	—	Chopi ...	—	?	?	Dr J. M. Collins.
May —	—	—	Unyoro	1 30 N.	31 45 E.	F. M. Isemonger.
" 20	—	Hoima...	Unyoro	1 25 N.	31 25 E.	C. L. Lea-Wilson.
July 13	—	—	—	?	?	Dr. C. Wiggins.

Description.—The male is a medium-sized tick of general red-brown colour, and with the scutum marked with indistinct whitish markings. The legs are long and red, without variegation. The female is much larger and of a dirty ochre-brown colour. The dorsal shield is small and confined to the anterior end, and is of a chocolate colour. The legs are like those of the male.

Bionomics.—Nothing is known of its habits, but it appears to be peculiar to the elephant. On the other hand these animals do not universally harbour the parasite, for elephants shot by Captain F. P. Mackie, I.M.S., in Ankole, in Toro, and in the Lado Enclave, were free from any ticks.

Amblyomma petersi.—This is found on the rhinoceros, the variety in each case being the small black rhinoceros found in Abyssinia and the Nile Province. Some specimens of this very handsome tick were forwarded by Dr. J. M. Collins, on March 6, 1909, from a rhinoceros killed in the Lango country, north of the Victoria Nile (2° 0' N., 32° 40' E.). Other specimens were secured by Captain F. P. Mackie, I.M.S., on two rhinoceroses shot by him near Koba, in the south of the Nile Province (2° 25' N., 32° 0' E.).

Description.—This is a very handsome tick of large size, is of a dark chocolate-brown colour, with large dorsal shields of a rich sulphur-yellow colour. There is less disparity between the size of the male and female than in some species, but the female is as usual the larger and possesses a smaller scutum than the male, and is less ornate. Nothing is known as to its habits, but it has not been found on any other animal than the rhinoceros.

Amblyomma sparsum.—Some specimens of this tick were caught on the road between Bussu and Bululu, in the locality of Namasala (0° 40' N., 33° 30' E.) by G. P. Jervoise, Esq.

Description.—The male is a very handsome tick of medium size, with a black dorsal shield ornamented with four or five paired markings of deep orange colour. The legs are dark-orange in colour, with bands of dark colour around the joints. The female is but little larger than the male, and has a body rather more elongated. The anterior portion of the body is overlaid with an orange-coloured shield, and the hinder portion is of dark colour ornamented with a pair of small yellow dots. The festoons, which in the male are of the same orange colour as the dorsal shields, are uncoloured and less conspicuous in the female.

Amblyomma splendidum.—Specimens of this tick were forwarded from four localities. In two cases where the host was named it was the buffalo.

Distribution in Uganda.—

Table 104.—*Amblyomma splendidum*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1909.							
Feb. 27	Mubango...	Chagwe ...	Uganda	0 35 N.	33 0 E.	E. Brown.	
Mar. 16	—	Bulemezi	„	0 35 N.	31 15 E.	Sir Apolo Kagwa.	
May 5	—	Unyoro ...	Western	1 25 N.	31 25 E.	F. M. Isemonger	Buffalo.
July 13	—	—	—	?	?	Dr. C. Wiggins ...	„

Description.—As its name implies, this is a very brilliantly-coloured tick. In the specimens to hand the female is smaller than the male, and the colouring in the two sexes is quite different. The male is variegated with chocolate-brown on a sulphur-yellow ground. The festoons are alternately yellow and chocolate. The legs are chocolate-brown banded with yellow. The female is of a brilliant green colour, with a small black or dark-brown shield. The legs are chocolate picked out with white, but are somewhat less ornate than those of the male.

Bionomics.—Its habits are unknown.

GENUS: RHIPICEPHALUS.

Rhipicephalus appendiculatus (Neumann).

The Brown Tick.

Native name: “Ngotia.”

Geographical Distribution.—This is very wide; it is one of the commonest ticks in Africa, and is probably nearly universal in distribution.

*Distribution in Uganda.—*Table 105.—*Rhipicephalus appendiculatus*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1908.							
Dec. 16	—	Kajingola	Uganda	0° 25' N.	32° 12' E.	Sir Apolo Kagwa.	
" 17	Namalinde	Bululu ...	"	0° 15' N.	32° 50' E.	" " " "	Cattle.
" 18	Mpumu ...	Chagwe ...	"				
" 23	—	Buzinga ...	"				
" 28	—	Butengese	"				
" "	—	Buddu ...	"				
" "	—	Bugerere	"	About		Sir Apolo Kagwa.	
				0° 25' N.	32° 12' E.		
1909.							
Jan. 1	—	Kabula ...	"				
" "	—	Mukifo ...	"				
" 4	—	Mawagola	—	0° 15' N.	31° 15' E.	The Kisingere.	
" 6	—	Buruli ...	"	1° 22' N.	32° 20' E.	Sir Apolo Kagwa.	
" 9	Mabrio ...	Koki ...	Uganda	1° 0' S.	31° 32' E.	" " "	
" 22	Lubembe	Bulemezi	"	0° 35' N.	32° 30' E.	" " "	
Feb. 23	Namu- kekera	"	"	0° 45' N.	32° 30' E.	Cpts. Bateman and Mackie.	
" 17	Mubango	Mabira ...	"	0° 35' N.	33° 0' E.	E. Brown.	
" 22	Entebbe ...	—	"	0° 04' N.	32° 26' E.	Director of Transport.	
Mar. 9	Sale ...	Gomba ...	"	0° 20' N.	32° 0' E.	Sir Apolo Kagwa.	
" "	Mamba ...	"	"	0° 20' N.	32° 0' E.	" " "	
" 16	Kibuze	Bulemezi...	"	0° 31' N.	32° 16' E.	" " "	
" "	Kiguli ...	"	"	0° 30' N.	32° 20' E.	" " "	
" "	Katonga river	Gomba ...	"	0° 05' N.	31° 40' E.	" " "	
" 28	Kezalizi ...	Mruli ...	Unyoro	1° 38' N.	32° 15' E.	F. F. Bloedow.	
Apr. 1	Kabaya ...	Mbarara ...	Western	0° 32' S.	30° 45' E.	Dr. A. C. Rendle.	
" "	Kabaka ...	"	"	0° 30' S.	"	"	
" 19	Mbale ...	Bukedi ...	Central...	1° 0' N.	34° 0' E.	Dr. J. A. Taylor.	
" 23	Mabira ...	Chagwe ...	Uganda	0° 35' N.	33° 0' E.	E. Brown.	
" 30	Mbalala ...	Lake Albert	Unyoro	1° 28' N.	31° 10' E.	L. C. Lea-Wilson.	
May 12	Kyetume	Chagwe ...	Uganda	0° 14' N.	32° 44' E.	Dr. R. van Somerens.	
June 6	Masaka ...	Buddu ...	"	0° 20' S.	31° 40' E.	Sir David Bruce.	
" 12	Mpumu ...	Chagwe ...	"	0° 15' N.	32° 50' E.	Commission.	
July 1	Khagora ...	Toro ...	Western	0° 40' N.	30° 20' E.	J. D. Buckland.	
" "	Mwarro ...	"	"	0° 40' N.	"	"	
" 3	Bululu ...	Bukedi ...	Central...	1° 40' N.	33° 20' E.	G. P. Jervoise.	
" 23	Sesse ...	Lake Victoria	Uganda	0° 30' S.	32° 10' E.	Dr. C. H. Marshall.	
" "	Ngolia ...	—	"	0° 34' S.	32° 10' E.	Col. L. C. E. Wyndham.	
" "	Lugoge ...	Bulemezi	"	0° 30' N.	32° 15' E.	Sir Apolo Kagwa.	
" "	Fort Portal	Toro ...	Western	0° 40' N.	30° 15' E.	Dr. Ashton Bond.	
" 26	Bussu ...	Usoga ...	Uganda	0° 30' N.	33° 30' E.	Dr. H. Bayon.	
" "	Nagongera	Budama ...	Bukedi	1° 20' N.	33° 30' E.	P. W. Perryman.	
" "	Kabulemu	Masaka ...	Buddu	About		D. L. Baines.	
" "	Kiangi ...	"	"	0° 20' S.	31° 40' E.	"	
" 31	Kabazi ...	Buruli ...	—	1° 10' N.	32° 10' E.	Sir Apolo Kagwa.	
Aug. 6	Mazigo ...	"	"	About		D. L. Baines.	
" "	Buzago ...	"	"	0° 20' S.	31° 40' E.	"	
" "	Masaka ...	"	"	"	"	"	
" 12	Kidongoli	Kumi ...	Bukedi	"	"	"	
" "	Kichum- bala	"	"	About		E. G. Morris.	
" "	Soroli ...	"	"	1° 40' N.	33° 20' E.	"	
" "	Magora ...	"	"	"	"	"	
" "	Malera ...	"	"	"	"	"	
" 17	Miro ...	Bululu ...	"	1° 40' N.	33° 15' E.	G. P. Jervoise.	
" "	"	N. Chioga	Bukedi...	1° 45' N.	33° 0' E.	" " "	
" 20	Buzugi ...	Sebondi's	Usoga ...	0° 55' N.	33° 40' E.	F. M. Isemonger.	
" "	Buziri ...	Wakoli's...	"	0° 30' N.	33° 45' E.	" " "	
" "	Bulembo...	"	"	0° 30' N.	33° 45' E.	" " "	
Sept. 14	Nami- rembe	Kiadondo	Uganda	0° 18' N.	31° 32' E.	C. J. Phillips.	Calves dy- ing of amakebe.
" 29	Mbarara ...	Ankole ...	Western	0° 30' S.	30° 45' E.	Dr. A. C. Rendle.	
Oct. 14	Jinja ...	Usoga ...	Uganda	0° 30' N.	33° 15' E.	Capt. G. Lane.	
" 18	Kitala ...	?	?	?	?	Dr. C. Wiggins.	

Description.—The Brown tick is very common and needs no special description. It is sombre and relatively inconspicuous, and is not characterised by any special markings. The male is small, and very active in its habits, and will frequently be seen travelling over the ground or crawling up the clothing at a considerable rate. It is uniformly reddish-brown. When touched it feigns death, drawing its legs under it, but quickly regains its activity when left unmolested. The male has its central festoons prolonged posteriorly, like the tail of a tortoise. The female is larger, and when distended is many times larger than the male. The dorsal shield is inconspicuous, and the general colour of the upper surface is of a dirty yellowish-brown and in the undistended condition is very wrinkled.

Bionomics.—It is necessary that this tick must find a warm-blooded host three times in its existence, namely as a larva, as a nymph, and as an adult. After each feed it drops off the host and undergoes metamorphosis on the ground, after which it awaits the coming of a new host. It is the principal agent in the transmission of East Coast fever (*Piroplasma parvum*). It transmits East Coast fever principally in its adult stage, having fed as a nymph on sick cattle, and less so in the nymphal stage, having become infected as a larva. It does not transmit the disease in its larval stage when born of an infected mother—that is, there is no hereditary transmission.

Rhipicephalus simus (Koch).

The Black-pitted Tick.

Distribution.—According to Howard, this tick is found widely in Africa, *e.g.*, German East Africa, Abyssinia, Sierra Leone, Transvaal, Cape Colony, Madagascar, and Mozambique. It seems, he adds, “to be confined to the more humid areas of South Africa. In Cape Colony it is confined more to the coastal belt of summer rainfall. In the Transvaal it seems to be most common in the bush and low veldt, and more rare in the south-west high veldt.”

Distribution in Uganda.—It did not appear to be common in Uganda, though owing to its close similarity to *Rhipicephalus appendiculatus* it may have sometimes been included with that species. Specimens were sent by G. P. Jervoise, Esq., from the Miro District (1° 45' N., 33° 0' E.).

Description.—The general appearance of this tick is very like that of *Rhipicephalus appendiculatus*, and without giving some minute details, it is difficult to describe obvious differences. It is, as its name implies, darker in colour and the dorsal surface is less shiny and more pitted.

Bionomics.—Its life-history is similar to that of *Rhipicephalus appendiculatus*. It feeds on almost any warm-blooded animal, particularly the herbivora. It assists in the transmission of East Coast fever (*Piroplasma parvum*), and probably also in that of *Piroplasma mutans*.

Rhipicephalus capensis (Koch).

The Cape Brown Tick.

This is a common tick in South Africa, and is believed to take part in the transmission of East Coast fever. Its occurrence was only verified twice in Uganda. Somewhere, taken from a buffalo shot by F. M. Isemonger, Esq., in the Province of Unyoro. Others were sent by Dr. J. M. Collyns, and were taken by him from the carcase of a wart-hog shot in Bulemezi, Uganda. In appearance it is like the two foregoing species: of a uniform red-brown colour without characteristic markings. The adult male appears to be larger than those of the last-named species. Howards gives cattle, goats, horses and dogs as the usual hosts.

Rhipicephalus evertsi (Neumann).

The Red Tick or the Red-legged Tick.

Native name: "Enkwa."

Geographical Distribution.—Howard gives the following: South Africa—Cape Colony, Transvaal, Natal, Rhodesia; German East Africa; tropical Africa; German South-West Africa; Mozambique.

Distribution in Uganda.—

Table 106.—*Rhipicephalus evertsi*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1903.							
Dec. 3	—	Ankole ..	Western	0 30 S.	30 30 E.	Sir Apolo Kagwa.	
—	—	Buddu ...	Uganda	0 15 S.	31 30 E.	"	
—	Lukokome	Koki ...	"	1 0 S.	31 32 E.	"	
—	Lubembe	Bulemezi	"	0 35 N.	32 30 E.	"	
1909.							
Feb. 4	Nakasetta...	"	"	0 35 N.	31 18 E.	"	
" 6	Quimba ...	"	"	0 35 N.	31 17 E.	"	
Mar. 9	Lake	Singo ...	"	0 25 N.	32 0 E.	"	
" 10	Wamala. Namuke- kera.	Bulemezi	"	0 45 N.	32 30 E.	L. C. Lea-Wilson.	Zebra.
" 11	Kimaga ...	Buruli ...	"	1 30 N.	32 30 E.	Sir Apolo Kagwa.	
" 16	Lukage ...	Bulemezi	"	0 30 N.	32 15 E.	"	
" 16	Kigule ...	"	"	0 35 N.	31 15 E.	"	
" 18	Bale ...	Buruli ...	"	1 5 N.	32 50 E.	"	
June 22	Mpumu ...	Chagwe ...	"	0 15 N.	32 50 E.	Commission ...	Goat.
July 26	Kiango ...	Masaka ...	"	0 20 S.	31 36 E.	D. L. Baines.	
Aug. 6	Buyango...	Buddu ...	"	0 22 S.	31 40 E.	"	
" 6	Masaka ...	Masaka ...	"	0 20 S.	31 18 E.	"	
" 6	Kabazi ...	Buruli ...	Uganda	1 22 N.	32 20 E.	Sir Apolo Kagwa.	
" 12	Kokoiyo ...	Bukedi ...	Central	1 48 N.	33 0 E.	E. G. Morris.	
" 17	Miro ...	"	"	1 45 N.	33 0 E.	G. P. Jervoise ...	
" 17	"	Bululu ...	"	1 45 N.	33 0 E.	"	Mule.
Sept. 28	Mbarara ...	Ankole ...	Western	0 30 S.	30 45 E.	Dr. A. C. Rendle.	

Description.—This tick in size and shape and general colouring resembles the other species of this genus—that is, it is a medium-sized tick, with a prevailing red-brown colour, without particular ornamentation. The male has yellow or saffron borders to the dorsal surface, and the legs are also of saffron-yellow colour. The female has no such distinctive colouration, and is of brownish colour, and when empty is deeply wrinkled. She is larger than the females of the *Rhipicephalus appendiculatus* and *simus*.

Bionomics.—Its life-history resembles that of *Rhipicephalus appendiculatus*, but "it is able to withstand very dry conditions, and is found even in the Karoo. It passes its first moult on the host and the second off the host—that is, it visits two hosts during its life-cycle instead of three. This tick has been found to be concerned to some extent in the transmission of East Coast fever; it also transmits biliary fever (*Piroplasma equi*) of the horse, mule, and donkey." Howard.

GENUS: BOOPHILUS (CURTICE).

SYNONYM: MARGAROPUS (KARSCH).

Boophilus annulatus, var. *decoloratus*.

The Blue Tick.

Native name: "Neuzo."

Geographical Distribution.—Howard states that this tick is distributed over nearly every part of Africa, and mentions the following places: South Africa (generally)—Cape Colony, Orange River Colony, Transvaal, Natal, Rhodesia; German South-West Africa; Madagascar; Mauritius; Congo; Uganda; German East Africa; Mozambique; Cape Verde Islands.

Distribution in Uganda.—This is very wide, as the following particulars show.

Table 107.—*Boophilus annulatus*, var. *decoloratus*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1908.							
Dec. 23	Buzinja ...	Bulemezi	Uganda	0° 40' N.	32° 30' E.	Sir Apolo Kagwa.	
" 28	Bugerere...	Chagwe ...	"	1° 0' N.	32° 50' E.	"	
" 28	Butengese	Buddu ...	"			"	
1909.							
Jan. 1	Musale ...	" ...	"	About 0° 0' & 1° 0' S. and 31° 0' E. & 32° 0' E.		}	"
" 1	Mududu ...	" ...	"				
" 4	—	Mawagola	"				
" 6	—	Buruli ...	"				
" 22	Nyondo ...	Bulemezi	Uganda	0° 15' N.	31° 15' E.	The Kisingere.	
" 22	Sambwe ...	"	"	1° 22' N.	32° 20' E.	Sir Apolo Kagwa.	
" 22	Bukeka ...	"	"	0° 33' N.	32° 16' E.	"	
" 22	Kyamboga	"	"	0° 35' N.	32° 30' E.	"	
Feb. 3	Namukekera.	"	"	About 0° 45' N. 32° 30' E.		Capts. Bateman and Mackie.	On a water buck.
" 9	"	"	"				
" 17	Mubango ..	Mabira ...	"	0° 35' N.	33° 0' E.	E. Brown.	
" 27	"	" ...	"	0° 35' N.	33° 0' E.	"	
Mar. 9	Sale ...	Gomba ...	"	0° 20' N.	32° 0' E.	Sir Apolo Kagwa.	
" 9	Lake Wamala.	Singo ...	"	0° 25' N.	32° 0' E.	"	
" 10	Namukekera.	Bulemezi	"	0° 45' N.	32° 30' E.	L. C. Lea-Wilson.	On zebra.
" 11	Kimaga ...	Buruli ...	"	1° 30' N.	32° 30' E.	Sir Apolo Kagwa.	
" 11	Kazwama	" ...	"	1° 20' N.	32° 40' E.	"	
" 11	Kiguli ...	" ...	"	1° 24' N.	32° 50' E.	"	
" 16	Lukage ...	Bulemezi	"	0° 30' N.	32° 15' E.	"	
" 16	—	On the Nile	"	1° 31' N.	32° 16' E.	"	
" 16	Katonga river.	Gomba ...	"	0° 05' N.	31° 40' E.	"	
" 16	Bale ...	Buruli ...	"	1° 5' N.	32° 50' E.	"	
Apr. 1	Mabira ...	Chagwe ...	"	0° 35' N.	33° 0' E.	E. Brown.	
" 1	Mbarara ...	Ankole ...	Western	0° 30' S.	30° 45' E.	Dr. A. C. Rendle.	

Table 107.—*Boophilus annulatus*, var. *decoloratus*—continued.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1909.							
Apr. 24	Jinja ...	—	Uganda	0° 30' N.	33° 15' E.	Capt. H. M. Tufnell.	
" 30	Mbalala ...	Lake Albert	Unyoro	1° 40' N.	31° 30' E.	L. C. Lea-Wilson.	
May 29	Sesse ...	Lake Victoria.	Uganda	0° 31' S.	32° 10' E.	Capt. A. D. Fraser.	
June 17	" ...	"	"	0° 33' S.	32° 15' E.	"	
" 22	Namirembe	Kiadondo	"	0° 18' N.	31° 32' E.	C. W. Hattersley.	
" 22	Mpumu ...	Chagwe ...	"	0° 15' N.	32° 50' E.	Commission.	
" 22	Seninja island.	Sesse ...	"	0° 30' S.	32° 20' E.	Capt. A. D. Fraser.	
" 22	Bumanja island.	" ...	"	0° 32' S.	32° 18' E.	"	
July 1	Khajora ...	Toro ...	Western	0° 30' N.	30° 15' E.	J. D. Buckland.	
" 1	Mwarro ...	" ...	"	0° 38' N.	20° 16' E.	"	
" 20	Kigule ...	Buruli ...	Uganda	1° 20' N.	32° 20' E.	Sir Apolo Kagwa.	
" 20	Dogola ...	" ...	"	1° 20' N.	32° 20' E.	"	
" 23	? ...	? ...	?	1° 20' N.	32° 20' E.	Col. L. C. E. Wyndham.	
" 23	Lugoge ...	Bulemezi	Uganda	0° 42' N.	32° 16' E.	Sir Apolo Kagwa.	
" 23	Junda ...	"	"	0° 40' N.	32° 38' E.	"	
" 26	Kanabulumu.	Masaka ...	Buddu ...	0° 22' S.	31° 38' E.	D. L. Baines.	
Aug. 6	Mazigo ...	" ...	" ...	0° 22' S.	31° 38' E.	"	Off Sesse cattle.
" 6	Buyago ...	" ...	" ...	0° 22' S.	31° 38' E.	"	"
" 6	Masaka ...	—	" ...	0° 22' S.	31° 38' E.	"	"
" 12	Kidongola	Bukedi ...	Central	—	—	E. G. Morris.	"
" 12	Kachumbala.	" ...	"	—	—	"	"
" 12	Soroli ...	" ...	"	1° 32' N.	33° 20' E.	"	
" 12	Bugiro ...	" ...	"	—	—	"	
" 20	Buzuge ...	Sebondi's	Usoga	0° 55' N.	33° 40' E.	F. M. Isemonger	
" 20	Nemeje ...	"	"	0° 56' N.	33° 40' E.	"	
" 23	—	Toro ...	Western	0° 30' N.	30° 15' E.	Sir Apolo Kagwa.	
" 26	Nagongera	Budama ...	Bukedi	0° 37' N.	33° 55' E.	P. W. Perryman.	
" 26	Bunyuli ...	"	"	0° 5' N.	33° 49' E.	"	
" 31	Kampala...	Kiadondo	Uganda	0° 20' N.	32° 35' E.	Director of Transport.	
" 31	Kabazi ...	Buruli ...	"	1° 23' N.	32° 20' E.	Sir Apolo Kagwa.	
Oct. 14	Keringia ...	Jinja ...	"	0° 25' N.	33° 15' E.	Capt. G. Lane.	

Description.—This is called the Blue tick on account of the colour of the distended female. The male is very small and insignificant, and of a reddish-brown colour, without distinctive pattern. The female is larger than the male, but never attains the size of the engorged females of the genus *Amblyomma*, and is generally smaller than those of the *Rhipicephalina*. The engorged females, which are about a quarter of an inch long, are commonly seen in paths and tracks in cattle country, looking like smooth bluish pebbles.

Bionomics.—These ticks are exceedingly prolific. They are said to give rise to several generations in a year. It is one of the most important of the pathogenic ticks, for it is responsible for the transmission of Texas fever in America, which disease in Africa, South America, and Australia is known as red-water fever, or bovine piroplasmiasis (*Piroplasma bigeminum*). It also carries a pathogenic spirillum (*Spirillum theileri* of cattle). *Piroplasma mutans* is not carried by any stage of the tick, nor is *Piroplasma parvum*, the parasite of East Coast fever. It attacks almost all the domestic animals, especially cattle, and it is also commonly found on wild game which feed over the same ground as the domestic herds.

GENUS: *HÆMAPHYSALIS*.*Hæmaphysalis leachi* (Audouin).

The Dog Tick.

Geographical Distribution.—Howard gives the distribution in Africa as follows: Egypt, Eastern Ethiopia, Abyssinia, Algeria, Sierra Leone, French Congo, Belgian Congo, Cameroon Mountains, Cape Colony, Orange River Colony, Transvaal, Mozambique, and German East Africa. From this it is apparently almost universal in distribution.

Distribution in Uganda.—Is shown by the following table.

Table 108.—*Hæmaphysalis leachi*.

Date.	Locality.	District.	Province.	Latitude.	Longitude.	Donor.
1908.						
Dec. —	—	Ankole	Western	0 30 S.	30 52 E.	Sir Apolo Kagwa.
„ —	Mpumu	Chagwe	Uganda	0 15 N.	32 50 E.	Commission.
1909.						
Apr. 19	Mbale ...	Bukedi	Central	1 0 N.	34 0 E.	Dr. J. A. Taylor.
June 16	Bussu ...	Usoga ...	„	0 32 N.	33 30 E.	Dr. H. Bayon.
„ 16	Sesse ...	Lake Victoria.	—	0 31 S.	32 10 E.	„
Aug. 12	Kumi ...	Bukedi	Central	1 30 N.	33 30 E.	E. G. Morris.

Description.—The male is very small and of a uniform reddish-brown colour, without distinctive markings. The female is larger than the male, and when engorged is of the colour of a ripe sloe—bluish purple.

Bionomics.—This is the common dog tick, and it confines its attentions to the carnivora, domestic or wild, and is rarely found on cattle even when the ticks are very abundant. It transmits *Piroplasma canis*, the distemper or malignant jaundice of dogs, a disease which is rapidly fatal to dogs in many parts of Africa.

FAMILY: ARGASIDÆ.

GENUS: ORNITHODOROS.

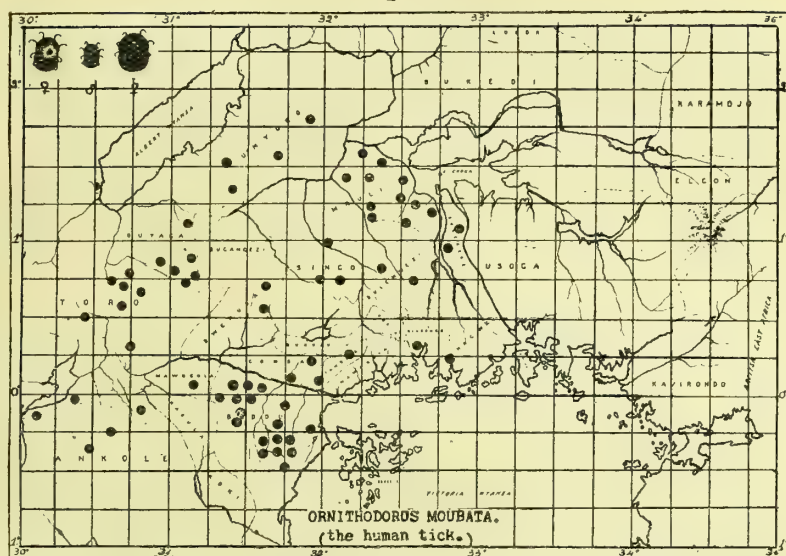
Ornithodoros moubata (Murray).—The carrier of human tick fever.

Native name: “Bibi.”

Geographical Distribution.—Howard gives the African distribution as follows: Somaliland, Kilimanjaro, Congo, Egypt, South-East Africa.

Distribution in Uganda.—This very important and much dreaded parasite is widely distributed throughout Uganda—probably much more so than the map shows. According to native reports it frequents nearly all the principal caravan routes, and in some places the huts have been so badly infected that all the rest-camps are periodically destroyed by order of the Government.

Map 10.

Table 109.—*Ornithodoros moubata*.

Date.	Locality.	District.	Province.	Lat.	Long.	Donor.	Remarks.
1908.							
Dec. 28	Kamaliya	Buddu	Uganda			Sir Apolo Kagwa.	
" "	Bugaga ...	"	"			" "	
" "	Kabanda...	"	"			" "	
" "	Kamurya	"	"			" "	
" "	Bugange ...	"	"			" "	
" "	Nakiojo ...	"	"			" "	
" "	Bulange ...	"	"			" "	
" "	Kalamba...	"	"			" "	
" "	Nsoziling	"	"			" "	
" "	Kigembo	Bugerere	"	1° 0' N.	32° 50' E.	" "	
" 31	Kabula ...	Buddu	"	0° 15' N.	31° 15' E.	" "	
1909.							
Jan. 1	Musale ...	Singo	"	0° 30' N.	31° 30' E.	" "	
" "	Mududu ...	"	"	"	"	" "	
" 4	Mawagola	Ankole	Western	0° 15' N.	31° 15' E.	The Kisingere.	
" "	Mwera ...	Busuju	Uganda	"	"	Sir Apolo Kagwa.	
" 6	Kirinidi ...	Buruli	"	"	"	" "	
" "	Munawo ...	"	"			" "	
" "	Kisalizi ...	"	"			" "	
" "	Kisali ...	"	"			" "	
" "	Nalukoge	"	"			" "	
" "	Nakotogo	"	"			" "	
" "	Kikonge ...	"	"			" "	
" "	Muwawo	"	"			" "	
" 11	Bugembe	"	"			" "	
" "	Bugon-	"	"			" "	
" "	gomle.	"	"			" "	
" "	Kagalwayo	"	"			" "	
" "	Wanane ...	"	"			" "	
" 22	Lubembe	Bulemezi	"	0° 35' N.	32° 30' E.	" "	
Apr. —	—	Toro	Western	0° 30' N.	30° 15' E.	Dr. A. Bond, " ...	Swarm in every house.
Dec. —	Many places on road from Kampala to Mbarara					Capt. F. P. Mackie.	
1910.							
Jan. and Feb.	The caravan roads from Mbarara to Fort Portal and thence to Hoima are infested with them. None was seen in the Nile Province.						

Description.—This tick belongs to the family *Argasidae*, which differ from that of the *Ixodidae* in one easily recognisable character: the rostrum, or biting apparatus, in the latter family projects anteriorly from underneath the body of the tick, whereas in the *Argasidae* this organ is concealed from view when the tick is seen from the dorsal surface. To take a familiar example, the common tortoise, when its head and neck are extruded, resembles the *Ixodidae*, but when the head and neck are withdrawn and invisible from above it resembles the family of *Argasidae*. The family of *Argasidae* are commonly parasitic on birds and bats, whereas the most important *Ixodidae* infest the bodies of the larger mammals.

The photographs on the distribution map give a good idea of the general shape and appearance of the tick. When young it is a light yellow or greenish-brown, and when older these colours fade to a dark green or brown. Its upper surface is studded with minute irregularities, or tubercles, which give it a rough shagreened appearance. The dorsal surface is of a regular oval shape, without any evidence of a head or mouth parts; the legs only project from under the body, so that when walking it fancifully suggests the Roman military formation known as the "Testudo." The male is about 7 mm. in length and the female about 13 mm.

Bionomics.—The habits of this tick differ from those previously described, in that it is a nocturnal feeder, and in that it feeds rapidly and does not attach itself for long to the body of its host. It rarely feeds on any other animal than man. It lives in the floor of old huts and sometimes in the mud walls or, more rarely, in the thatch. Its habits are very like those of the bed-bug: it appears in the evening or at night, feeds from the bodies of those who are asleep in the hut, and after feeding crawls away to hide in the cracks or crevices of the mud floor. It leaves a small circular weal after biting; irritation commences soon after the bite, and lasts a few hours. There are no other local signs. It gives rise to spirillum fever, or tick fever, in man—a very severe fever, characterised by four or five relapses, which last sometimes for many weeks, and followed by very severe after-effects. The only way to avoid the bites of these ticks is to clear a new camping ground every night, and on no account to use old huts or places cleared for camps. Porters should never be allowed to take bedding into a hut by the road side, even to shelter from the rain, and they should never be allowed to carry their own bedding attached to their master's baggage, as they are so fond of doing.

Ornithodoros savignyi (Audouin).

This tick is a very closely related species to *Ornithodoros moubata*, and generally replaces that variety in many parts of South Africa. It is believed to transmit human tick fever. Only one specimen was received by the present Commission. It was forwarded by G. P. Jervoise, Esq., from the Miro District, in Bukedi, in the Central Province of Uganda (1° 45' N., 33° 0' E.).

In addition to those ticks above described, a few others were found which possess scientific rather than economic interest.

One was a spotted tick caught on an iguana, brought up from the shore of Lake Victoria. This parasite is that known as *Aponomma exornatum* (Koch) (the lizard tick), and is peculiar to the reptilia. One tick, probably of the same species, was found also on a python. Another species was found infesting the scaly ant-eater.

J. DISTRIBUTION OF CATTLE DISEASES IN UGANDA.

45.—THE DISTRIBUTION OF CATTLE DISEASES IN UGANDA.

In June, 1909, a study of the distribution of the various cattle diseases in Uganda was undertaken by the Commission. The help of the officials, missionaries, and settlers was solicited for this investigation. The Commission are greatly indebted to these gentlemen* who so courteously furnished reports and maps of their districts, and beg to thank them for the care and trouble which have been taken in their compilation.

The Acting Chief Secretary to the Uganda Government (Mr. A. G. Boyle, C.M.G.) kindly forwarded the following circular letter to the various officials in Uganda. Copies were also sent by the Commission to the missionaries, traders, settlers, &c., of Uganda:—

“ Sleeping Sickness Commission of the

“ Royal Society,

“ Mpumu, Chagwe, Uganda,

“ June 15, 1909.

“ SIR,

“ As I am anxious to work out the distribution of the various cattle diseases in Uganda, I would be greatly obliged if you would kindly answer the following questions, so far as you are able:—

- “ 1. Is your district, on the whole, a healthy one for cattle?
- “ 2. What, roughly, is the number of cattle in your district?
- “ 3. What is the percentage of loss from cattle disease per annum?
- “ 4. Is there any part, or parts, of your district which is unhealthy for cattle?
- “ 5. If unhealthy, is there any reason to be given for its unhealthiness?
- “ 6. What are the physical features of the unhealthy parts?
- “ 7. Is there any seasonal prevalence of the disease?
- “ 8. What diseases have you among the cattle of your district?
- “ 9. What are the native names of these diseases?
- “ 10. Do the natives attribute the disease to any blood-sucking insect?
- “ 11. Would you kindly make a rough tracing of your district, marking the healthy and unhealthy parts: A red cross for healthy, and a black cross for unhealthy?

* The names are given in the Appendix to this paper.

“ 12. Would you ask the chiefs to send in collections of biting flies and ticks which attack the cattle? And when sending them, to give the latitude and longitude and name of place where they were collected: Whether the place is healthy or unhealthy for cattle, and whether the natives attribute any disease, and what disease, to the bite of any insect?

“ 13. Have you a disease called “amakebe” among calves in your district?

“ 14. Is this disease restricted to certain parts, or does it occur everywhere?

“ Any information that you may be good enough to communicate, and which is used by the Commission, will be fully acknowledged.

“ I am, Sir,

“ Your obedient Servant,

“ (Signed) D. BRUCE, Colonel,

“ Director, Sleeping Sickness Commission.”

A summary of the answers received to the queries in the above circular letter will be given below, each answer under its own question.

QUERY 1.

Is your district, on the whole, a healthy one for cattle?

From the information obtained in answer to Query 1, it would appear that the unshaded areas on the accompanying map (*see* p. 248) are, on the whole, healthy for cattle. Some of these unshaded areas, whilst they are healthy for cattle bred in the district, are unhealthy for imported oxen. For example, the sub-districts of Egara and Kazara, on the west of Ankole, are healthy for local cattle, but are said to be unhealthy for cattle brought in from adjoining sub-districts (Mr. A. H. Watson, District Commissioner). Again, in the Nile Province, Mr. R. D. Anderson, District Commissioner, at Nimule, for the district between the Uma River on the north and the Zoka River on the south, states that this country is healthy for cattle bred in the district, but is unhealthy for imported cattle.

QUERIES 2 AND 3.

What, roughly, is the number of cattle in your district?

What is the percentage of loss from cattle disease per annum?

It had better be stated here that for the purposes of administration, the Uganda Protectorate is divided up into Provinces. The Provinces are:—

1. The Kingdom of Uganda, comprising Uganda proper.
2. The Western Province, consisting of Ankole, Toro, and Unyoro.

3. The Central Province, comprising Busoga, Bukedi, and the Elgon District.
4. The Nile Province, composed of the Nile, Bari, and Nimule Districts.

The following table summarises the replies given to these two queries:—

Table 110.

Province.	Approximate No. of Cattle.	Approximate percentage of annual loss.	Remarks.
Uganda Kingdom	85,805	16-26 per cent.	Estimated for year 1908.
Ankole	75,000	10 "	
Toro	5,776	10 "	
Unyoro	1,300	20 "	
Bukedi	100,000	10 "	
Busoga	50,000	10 "	The percentage is that given for Gabula, the most healthy district of Busoga.
Elgon district ...	150,000	15 "	Estimated only for Nile district.
Nile Province ...	13,400	5 "	

Remarks.—It is seen that out of a total of 481,281 head of cattle available for statistical purposes in the Uganda Protectorate, there is an annual loss of 12 per cent. at least.

Further interesting information on these questions is supplied by the Regents for the Kingdom of Uganda, through Mr. F. A. Knowles, C.M.G., Provincial Commissioner of the Uganda Kingdom (in 1909). He states, in Buddu District, out of 1,775 cattle, 9 per cent. were lost during the months of January, February, and March, 1909. During the same short period there died, in the Gomba District, out of 514 cattle, 8·5 per cent.; in Bulemezi, out of 1,009 cattle, 9 per cent.; in Buruli (Mruli), out of 1,055 cattle, 10·4 per cent.; in the Singo District, out of 320 cattle, 8 per cent.; and in Chagwe, out of 47 head, 4 per cent. were lost.

Mr. D. L. Baines, District Commissioner, at Masaka, in 1909, states that the Buddu District supports a total of, approximately, 26,000 head of cattle, and that there is an annual death-rate of 12 per cent.

QUERY 4.

Is there any part, or parts, of your district which is unhealthy for cattle?

The unhealthy districts, stations or villages, which are specially mentioned in the reports as being unhealthy for cattle, are given below. Reference should also be made to the map at p. 248; the unhealthy areas are shaded in this map.

The Uganda Kingdom.

The Kyadondo District is stated to be very unhealthy throughout for cattle. Kampala (Mengo) Station, and a radius of about six miles around the station, are notoriously unhealthy for calves and cattle. This locality is a hotbed of "amakebe," from which 60 to 75 per cent. of the calves are said to die annually. The cattle suffer from trypanosomiasis (*Trypanosoma pecorum* infection) to some extent, especially those oxen which travel between Luzira, the port of the town, and the town itself. The Namukekera Rubber Estate, to the west of and adjoining the Myanja River, suffered severe losses amongst its herd of draught oxen, in 1909, owing to periodical outbreaks of trypanosomiasis, the infecting agents being *Trypanosoma pecorum*, *Trypanosoma nanum*, and *Trypanosoma vivax*. The Chagwe District supports but few head of cattle, owing to its unsuitable pasture. In this district the old telegraph road between the Mission Station of Namityango and Kyetume Sleeping Sickness Camp, and the Lake-shore areas of the district, are dangerous for cattle. The Mission Station at Nkokonjeru, and Kasozi Village, in this district, are also stated to be unhealthy. Cattle travelling over the road between the Mabira Forest and the ford on the Nile opposite Jinja, occasionally contract trypanosomiasis, caused usually by an infection of *Trypanosoma pecorum* or *Trypanosoma vivax*. In this neighbourhood a few cases of nagana (*Trypanosoma brucei*) have been detected. Entebbe and the Entebbe-Kampala Road are unhealthy for cattle. Large numbers of Government transport cattle died on this road and in the town in 1908-9 from trypanosomiasis caused by *Trypanosoma pecorum* infection. The Gomba District is variously stated as healthy and unhealthy for cattle. The district has been for many years the grazing country of Uganda. Formerly healthy, it appears of late to be getting less healthy each succeeding year. This is attributed to the importation of unhealthy stock from other districts and to in-breeding. The only village particularly mentioned as unhealthy is Kissozi, on the Katonga River. In the Butambala District the villages of Kabuli and Kabasanda are given as unhealthy, and in the Mawokota District, Kantinyi Village is similarly mentioned. The Bwekula District is stated to be generally unhealthy for cattle, with the villages of Butorogo and Kiruma, in the north, and Kawanga, in the south, as its most unhealthy spots. Bugangezi District is also unhealthy throughout, the village of Bukumi being its most unhealthy part. Father Lafleur, of the White Fathers' Mission, reporting on the Bwekula and Bugangezi Districts, states that at Bukumi in the year 1908 there was a 40 per cent. loss of cattle, and in 1909, 50 per cent. were lost at the same place. At Butorogo, in the Bwekula District, he writes, that 80 per cent. of the cows and 40 per cent. of the calves were lost in 1908. The district of Buddu is, on the whole, a healthy one, the sub-districts of Bwela, Katumba, and Buluala being especially favourable for cattle. The unhealthy areas in this district are the Missionary Station at Villa Maria, Masaka, and its vicinity, and a strip of land

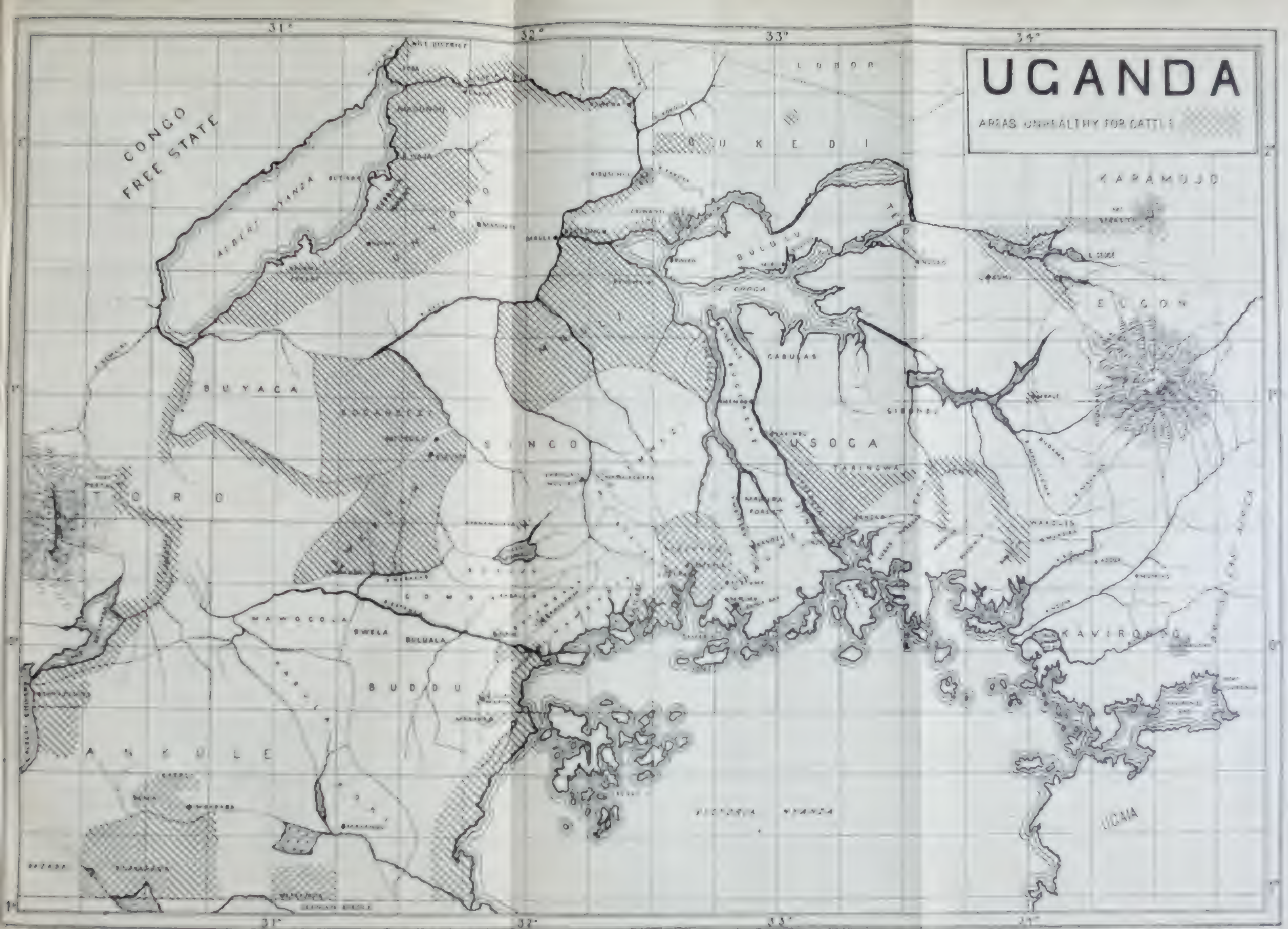
some 20 miles broad bordering the Lake-shores of this country. In the sub-district of Koki the village of Mayango would appear to be the only unhealthy spot. The village of Busuabwera, on the Katonga River, in the sub-district of Bwela, is also dangerous for oxen. The country of Buruli (Mruli), to the north of the Uganda Kingdom and bordering Lake Chioga, is stated by the Regents to be unhealthy for cattle of late years. Many cattle formerly grazed in this country. During the past few years there has been a heavy mortality amongst the stock—10·4 per cent. during the three months of January, February, and March, 1909. The vicinity of Kyulwe Village, near Lake Chioga, is given as the most unhealthy part of this country. The country of Bugerere, to the north-east of the Uganda Kingdom, does not appear to be a cattle country, as it supports but few head. Its northernmost point on Lake Chioga, and especially the villages of Bweyale and Kigembo, are said to be specially unhealthy.

Two interesting facts are emphasised in these reports. Firstly, that important townships (such as Kampala, Entebbe, Masaka), large missionary stations (such as Villa Maria), and villages on main roads of cattle traffic are all unhealthy for cattle and calves. "Amakebe," a disease of calves, which the Commission believe to be East Coast fever, is constantly present in these spots. This disease is very fatal to calves, causing a death-rate varying from 25 to 75 per cent., according to the care and treatment given to the sick. The second fact brought to light is the unhealthiness of the Lake-shore districts of the Victoria Nyanza. This is probably due to the presence of tsetse flies (*Glossina palpalis*). The Commission have proved that these flies are able to transmit the two most common and most fatal cattle trypanosomes in the Kingdom of Uganda, viz., *Trypanosoma pecorum* and *Trypanosoma vivax*. Mr. M. Moses, writing from Kampala, in 1909, states: "The Lake-shores are distinctly unhealthy; further inland it is not so bad. The cattle are tormented by innumerable flies I think the tsetse fly is the chief cause of unhealthiness." Mr. Moses, who has had many years of experience in dealing with cattle throughout the whole Protectorate, also believes that the *Stomoxys* and other biting flies may worry cattle so much by their bites, that the animals are prevented from grazing, and so get thin and die.

Ankole.

The Kingdom of Ankole, of the Western Province, is on the whole a healthy country for cattle; it supports some 75,000 head. The sub-district of Ngarama in the south, on the German border, is the most unhealthy area in Ankole. The districts of Kasali, Sema (Shema), Ruampara, and Bunyaruguru are also reported unhealthy, but to a less degree, imported cattle being the chief sufferers in these districts. The Kazara sub-district, to the extreme south-west of Ankole, is stated to be healthy for locally-bred cattle, and unhealthy for imported stock. The township of Mbarara, the official headquarters of the country, is very unhealthy for oxen, cows, and calves.

AREAS UNHEALTHY FOR CATTLE:



Toro.

The Kingdom of Toro, of the Western Province, was formerly a healthy country for cattle; of late years, however, there has been a considerable increase in the cattle diseases of the country. These diseases Mr. J. de G. Delmege, Assistant District Commissioner of Toro, reports are now widespread. Toro supports only 5,776 head of cattle at the present time (1909), and there is an annual loss of 10 per cent. amongst these. Fort Portal (Gerry) and its vicinity, the banks of the Mpanga River, the eastern shores of Lake Ruisamba (Lake George), Fort George, and practically the entire length of the northern boundary of the country, are mentioned by Mr. Delmege as being particularly unhealthy for cattle. The tsetse fly, *Glossina palpalis*, ranges along the eastern shores of this Lake and along the rivers flowing westward into the Lake. In the wooded country to the north of the Mpanga River, lying between the river and the Lake, and the forest to the east and south-east of Fort Grant on the borders of the Lake, *Glossina fusca* are present. A fine road from Entebbe traverses the country from west to east, terminating at Fort Portal (Gerry). The traffic along this road from such an unhealthy spot as Entebbe is possibly responsible for the rapid introduction of disease into this Kingdom. The disease "amakebe" is well-known to follow such routes. The natives of the country attribute much of the sickness of the cattle to the bites of a fly called locally "Kabugo" (genus and species unknown). This fly, they state, conveys disease from the wild game, especially the giant wild pig ("Nsenje") and the water-buck, to the cattle by its bite.

Unyoro.

The Kingdom of Unyoro, of the Western Province, is an unhealthy one for cattle. It supports only 1,300 head, amongst which there is an annual loss of 20 per cent. Captain H. M. Tufnell, Assistant District Commissioner, at Hoima, reports that the most unhealthy districts for cattle in Unyoro are Jemusi's Country, Chabigambi, Buswerkera, Bugumola, Butoli, Palangasa, and the township of Hoima. Mr. M. Moses states that the fords at Foweira and Fajao, on the Victoria Nile, are very unhealthy for oxen. In 1896 he lost cows at Fajao, and was informed that cattle could not live at this place. When at the same place, in 1899, he found that there was a mortality of 80 per cent. amongst the cattle of native chiefs at this ford and its vicinity. This loss was attributed by some of the natives to the bites of the tsetse fly; by others to the poor grazing. Owing to the presence of *Glossina palpalis* at Foweira and Fajao, these fords are now closed to traffic. Mr. C. F. H. Henry, Assistant District Commissioner, at Masindi, and the Reverend H. W. Tegert, write that Masindi Station and its neighbourhood, and those parts of the Fajao Road where tsetse fly are found, are unhealthy. *Glossina palpalis*, *Glossina morsitans*, and *Glossina pallidipes* all occur in the country to the south and east of Fajao. The sub-district of Magungu is also unhealthy throughout. A

considerable area of the unhealthy parts in the west of Unyoro is occupied by the two very large and dense forests of Budonga and Bugoma. Many biting flies are said to infest these forests and possibly account for their unhealthy reputation. In Map 1, "Sleeping Sickness and *Glossina palpalis* in the Uganda Protectorate," published with the first "Half-yearly Report of the Sleeping Sickness Extended Investigation, 1906," *Glossina fusca* are shown to be present in both the Budonga and Bugoma Forests; *Glossina palpalis* also occurs along the Waja River, the northern boundary of the Budonga Forest, and along the majority of the rivers flowing westwards into Lake Albert through these forests.

Usoga (Busoga).

The country of Busoga, in the Central Province, was formerly rich and prosperous. Sleeping Sickness and the great famine of 1907-8 have considerably reduced the population and its resources during the past few years. The wealth of the natives throughout Uganda is largely represented by cattle, so that the numbers of these must have been considerably reduced in Usoga as a result of disease and famine. At the present date (1909) the country supports about 50,000 head of cattle. Statistics of the annual mortality are not available for the whole country. In the sub-district of Gabula, the most healthy cattle district in Usoga, the annual loss is reported to be 10 per cent. (Mr. F. M. Isemonger, Provincial Commissioner, and Dr. H. B. Owen, Uganda Medical Staff, of Jinja). These officers give detailed statements of each sub-district. The following statements are taken from their report:—

Lusobyga.—Generally very unhealthy. Lack of good grazing, the presence of much elephant grass and forest, and the prevalence of biting flies, especially the "Mbwa" fly (*Simulium damnosum*), are given as reasons for the unhealthiness of this sub-district. It may be added that tsetse fly abound along the east banks of the Nile between Jinja and Kakindu.

Luba's District.—Very healthy for cattle, as there is good short grass, a plentiful supply of water, and no "Mbwa" fly. It is interesting to note that *Glossina palpalis* and also the "Kawawa" tick are plentiful in this Lake-shore country.

Naniumba's.—Is also a very healthy country for cattle. Gabula (Budioppe), to the north-east of Usoga, is reported to be the healthiest cattle district in Usoga: like Luba's District, it provides excellent grazing, is well watered, and is free from the "Mbwa" fly. For the same reasons Wakoli's and Mumia's to the west are, on the whole, healthy districts; the inland parts away from the Mpologoma River being unhealthy, as there is much elephant grass in these parts, and the "Mbwa" fly are present in large numbers.

The Sibondo (Semondo) sub-district in the north-west of Usoga is healthy. The Tapingwa sub-district is healthy in its northernmost parts and unhealthy elsewhere.

Elgon District.

This district is a very healthy one for cattle. It supports 150,000 head. The annual mortality is 15 per cent. Mr. H. P. Wright, Assistant District Commissioner, at Mbale, reports that Mbale and a radius of five miles around the township is the only unhealthy spot for cattle in the whole district. This area, he states, has a very heavy annual mortality (85 per cent.), and is especially dangerous to cattle imported from Bukedi and the neighbouring slopes of Elgon. The Reverend A. L. Kitching, reporting on the Teso country to the north and north-west of the Elgon District, writes that this country is, on the whole, healthy, so far as is known. The unhealthy parts comprise strips of country bordering the north and south shores of Lake Salisbury, an especially bad strip bordering Lake Chioga (Kyoga) to the south of the Agu River, and the stations of Ng'ora and Kumi. This country contains from 8,000 to 10,000 head of cattle owned by native chiefs.

Bukedi (Lango).

This country lies to the north of Lakes Chioga and Kwania. On the west it is bounded by the Victoria Nile. Bukedi supports some 100,000 head of cattle; the annual mortality amongst these is 10 per cent. These figures are only approximate. Mr. R. Paske Smith, Assistant District Commissioner, at Palango, reports on the district which lies along the eastern banks of the Victoria Nile, from the Tochi River in the north to the north-western shores of Lake Chioga in the south. He states he is informed by the natives that cattle which are grazed on the banks of the rivers Parosa, Koli (Kokolli), and Tochi, keep healthy; if removed from these rivers they die. A strip of country some 10 miles broad to the east of the Nile, from Kibusi Hill near the mouth of the Parosa River to Lukungu Village in the south, and from Lukungu eastwards along the shores of Lake Chioga to Chiawante Hill, are the most unhealthy areas in this country. This area is covered with thick scrub, and is waterless. *Glossina morsitans* are found throughout the scrub. The Bululu sub-district of Bukedi is reported upon by Mr. G. P. Jervoise, Assistant District Commissioner. This sub-district lies for the most part between Lake Chioga to the south and Lake Kwania to the north. It is healthy for cattle.

The Nile Province.

This Province is divided up into three districts for administrative purposes: The Nile, Nimule, and Bari Districts. These districts support some 13,400 head of cattle: 10,000 in the Nile District, 2,000 in the Nimule District, and 1,400 in the Bari District. The annual mortality amongst these cattle is not known, except in the Nile District, where it is 5 per cent. Mr. P. T. Hannington, Assistant District Commissioner, of the Nile District, reports that his district is, on the whole, a healthy one for cattle. He mentions as unhealthy parts of the country the immediate vicinity of the Assua River, and the Akuma Forest, which lies in

the triangular piece of country of which the White Nile and the Zoka River form the two sides. Three species of tsetse fly are found in this district. *Glossina palpalis* ranges along the entire length of the northern banks of the Victoria Nile and its tributaries flowing from the north. It is also to be found along the eastern banks of the White Nile, and the banks of the Tongi (Tengi), Assua (Achwa), Umi (Uma), and Zoka (Zukar) Rivers. *Glossina palpalis* and *Glossina morsitans* are present in the Akuma Forest, in the north-west corner of this district, and again in the south of the country in a strip of land just to the north of the Victoria Nile, opposite Fajao. The distribution of the tsetse fly thus corresponds fairly accurately with the unhealthy parts of this district. Mr. R. D. Anderson, District Commissioner, reporting on the Nimule District, states that this country is healthy for all but imported cattle. He mentions two parts of this district as being specially unhealthy for cattle: the northern banks of the Zoka River and its vicinity, and Affundo (Appundo's). *Glossina palpalis* and *Glossina morsitans* occur in both these neighbourhoods. Mr. E. B. Haddon, Assistant District Commissioner, of the Bari District, states that this district is not healthy for cattle. Formerly (about 1860) the natives possessed many cattle, and the country, especially in the neighbourhood of Gondokoro, was more under cultivation than it is at the present time (1909). Between 1860 and 1890 slave traders decimated the population and the herds, with the result that areas formerly under cultivation reverted to bush country, which soon harboured the tsetse fly and wild game, elephant, buffalo, and buck becoming numerous. At the present time, this district is covered, for the most part, with bush and high grass. The majority of the population, cattle-owning and otherwise, live along the banks of the Nile, as the inland rivers dry up in the dry season. For the same reason, in the dry season, the game collect along the river Nile, the Rivers Kit and Uma, Lake Birika (north of Gondokoro), and a small lake east of Gondokoro. The vicinity of these two lakes and the country to the east of Gondokoro is said to be fatal to cattle. The tsetse flies, *Glossina morsitans* and *Glossina pallidipes*, occur throughout this unhealthy country, and also in the bush south of the Kit River, which Mr. Haddon states is unhealthy. The eastern banks of the River Nile from Gondokoro to the Uma River and the country in the vicinity, would appear to be healthy for cattle in years with a good rainfall, and unhealthy in dry years. From the Uma River in the south near Forki Hill the Nimule-Gondokoro cart road traverses this district in a north-north-west direction to Gondokoro. *Glossina morsitans* appears to range along the entire length of this road and its immediate vicinity, with the exception of that part of the road which runs from a few miles north of Forki Hill to the village of Kiriloo. Cattle are kept at Tombe Musa to the west of the road in the sub-district of Kenni: their health is variable, good in some years, bad in others. There are no tsetse flies apparently in this spot. The various islands in the Nile, especially those near Gondokoro, are favourite grazing grounds in the dry season, the cattle appearing to thrive and keep healthy on them.

QUERY 5.

If unhealthy, is there any reason to be given for its (a district's) unhealthiness?

From the replies received in answer to this question, the following reasons are most commonly given for ill-health and disease amongst the cattle in the Uganda Protectorate:—

1. The presence of biting flies in a district.
2. The presence of certain wild game in a district.
3. Unsuitable grazing.
4. The movements of cattle from one district to another.
5. In-breeding.

1. *The Presence of Biting Flies in a District.*—The biting flies which have been associated with the occurrence of ill-health and disease amongst the cattle in Uganda are the “Bivu” (tsetse), the “Mbwa” (*Simulium damnosum*), the “Kawawa,” and “Nswera” (*Stomoxys*), the “Dumambogo” (*Tabanidæ*), and “Kabugo” flies.

The Tsetse Fly.—There are at least four different species of tsetse fly in the Uganda Protectorate—the *Glossina palpalis*, *Glossina morsitans*, *Glossina pallidipes*, and *Glossina fusca*. The different localities in which these flies are to be found have been detailed above in answer to Query 4, and it has been shown that, with but few exceptions, those parts of the country where tsetse flies have been found are reported to be unhealthy for cattle. Mr. M. Moses thinks that the tsetse fly is the chief cause of unhealthiness amongst cattle, and the reports from Unyoro, Toro, and the Nile Province endorse his opinion. Full-grown oxen are referred to, not calves. The co-existence of cattle disease and tsetse fly in a district must not, however, be taken as proof that the fly is responsible for the disease. In Luba's sub-district, in Usoga, an instance is given where *Glossina palpalis* and cattle live side by side without, apparently, producing disease amongst the stock.

The “Mbwa” Fly (*Simulium damnosum*).—The reports from Usoga (Busoga) District, in the Central Province, are almost unanimous in associating this fly with ill-health amongst cattle. The word “ill-health” is used in preference to disease, as this fly by its numbers and voracious habits, worry the grazing cattle to such an extent that the animals are unable to properly feed and rest during the day. The fly is more prevalent in this district than in any other part of the Uganda Protectorate.

The “Kawawa” or “Nswera” Fly (*Stomoxys*).—This fly is widespread throughout the Uganda Kingdom. It is especially prevalent in the larger towns and villages, where swarms of this insect are a constant source of irritation to the cattle throughout the sunny hours of the day. In the worst areas the herds obtain but little rest or food and soon become thin and ill-conditioned, and may even die. Mr. Moses emphasises the harm done to cattle by this fly. He writes: “What I want to impress on the Commission

is, that I believe the ordinary house-fly* has as much to do with the deaths of cattle about Kampala as the tsetse. They worry them to death by continual bites." In the Uganda Kingdom the *Stomoxys* is called either "Kawawa" or "Nswera."

The "*Dumambogo*" Fly (Tabanidæ).—The three principal genera of the family Tabanidæ found in Uganda are the *Tabanus*, *Hæmatopota*, and *Chrysops*. The native name "Dumambogo" probably includes the different species of these genera. The Tabanidæ are sometimes called "Bivu," or "Kivu," though these names are more commonly reserved for the tsetse fly, *Glossina palpalis*. Natives know that these flies feed on cattle and are a serious source of irritation and worry to them. For this reason the native herdsmen try to avoid their haunts when possible. From the reports received these flies do not appear to be associated with the transmission of disease. The distribution of the different species of the above genera is so widespread in the Uganda Protectorate, that the reader is referred for further information on this point to the Commission's paper entitled "The Distribution of Biting Flies in Uganda."†

The "*Kabugo*" Fly.—This native name is not known to the Commission; the genus and species of the fly are therefore not known. It occurs in Toro, in those parts of the country which are unhealthy for cattle, and is stated by the natives to transmit disease from the wild game, especially water-buck and giant wild pig, to the oxen. Country covered with forest and elephant grass would appear to be its favourite habitat. It has been pointed out above that this type of country, in the east of Toro, harbours the tsetse flies, *Glossina palpalis* and *Glossina fusca*; the native name may therefore refer to these flies.

2. *The Presence of Certain Wild Game in a District.*—Wild game and cattle disease are associated in the reports from several parts of the country in the Uganda Protectorate. This association in the Toro country has already been referred to. In the Bari District of the Nile Province, the natives especially connect elephant and buffalo with cattle disease. They believe that the dung of these animals poisons the grass, and that cattle eating this grass die. In the Nile District of the same Province, a fatal cattle disease called locally "Sadow" is also attributed to the presence of game. The natives say that it is caused by cattle "feeding in the footsteps of game." In the waterless country covered with thick scrub in the south and west of Bukedi, the natives associate the rhinoceros with cattle disease; indeed, their two most fatal and most common cattle diseases are called "Pyoko" and "Kulu," which mean literally rhinoceros. In the districts mentioned above three species of tsetse fly occur; in the Nile and Bari Districts *Glossina palpalis*, *Glossina morsitans*, and *Glossina pallidipes*; in the Bukedi scrub country *Glossina morsitans* and *Glossina fusca*. The association of tsetse fly, wild game, and cattle disease is thus clearly shown.

* *Stomoxys* is very like the house-fly, but is a biting fly and quite distinct.

† See p. 195, of this Report.

3. *Unsuitable Grazing.*—The Kiadondo District is unhealthy throughout on account of its unsuitable grazing, large areas of the country being overgrown with coarse elephant grass. Reports from other districts give a similar reason for the unsuitability of tracts of country.

4. *The Movements of Cattle from one District to another.*—Frequent mention is made in the reports of the ill-health and disease which follows the movement of cattle from their own district. Mr. Moses writes: "I believe cattle of certain districts do not stand shifting from their homes. Among these notably are the Ankole, and what are called the Bukedi cattle." Mr. Jervoise, Assistant District Commissioner, writing from Bululu Miro, in Bukedi, states: "What are called Bukedi cattle, viz., those from the Mbale and Kumi Districts, are said to be of little value when transported to Buganda (the Kingdom of Uganda) and Bunyoro (Unyoro), except for purposes of slaughter. They are said to get ill and die within a month or two of being moved." This information he writes was given him by the natives of the country. It bears out what Mr. Moses has said. Several suggestions have been made to account of this ill-health and disease which is associated with the movement of cattle. In Ankole there are many salt-licks, and the streams are stated to be slightly brackish; and both the cattle and the game are big-horned in this country. The lack of these salt-licks, the change of grass, and the climatic changes, probably form a combination of circumstances which account for the ill-health of these cattle when moved from their own country. Bukedi cattle in their journey to the Kingdom of Uganda and Unyoro have to travel through Busoga, where they are tormented by the "Mbwa" fly; they also pass through unhealthy stations, and are at several spots exposed to the bites of the tsetse fly, *Glossina palpalis*. These facts, combined with the changes in climate and fodder, may account for the reputation they have obtained.

5. *In-Breeding.*—The Reverend T. B. Fletcher attributes the increasing prevalence of disease and ill-health in the Gomba and Singo Districts to in-breeding. He reports that for some years past no new stock has been introduced into the country. Owners of stock buy and sell very locally. In former days the stamina of the stock was kept up by the introduction of new blood from the adjoining countries, the outcome of raiding and wars. As a result the present breed of cattle have but little power of resisting disease, changes of climate, &c. He states that native owners sometimes use a bull to serve his own stock to the second and even the third generation. No mention is made of this factor in any of the other reports.

QUERY 8.

What are the native names of these diseases?

A list of the diseases met with in each district of the various Provinces of the Protectorate was sent to the Commission in reply

to this query. The diseases most commonly reported and a brief outline of their symptoms are given herewith:—

The Uganda Kingdom.

The diseases most commonly reported from this Province are:—

1. “*Amakebe*” (*M’kebe*).—A disease of calves characterised by swelling of the glands of the neck, fever, and emaciation. High mortality.

2. “*Nsotoka*” (*Bwizulanda, Bahima*).—Said to be a lung disease, probably pneumonia, and highly infectious, one infected animal infecting a whole herd. The Reverend F. Rowling thinks it is probably rinderpest, and states that in the Kiadondo District epidemics occur about every ten years. Very fatal.

3. “*Kamenyo*” (*Kumenya*).—Gradual loss of strength, with emaciation. Finally animal falls down, is unable to rise, and death follows in a few days.

4. “*Koboa*” (*Kabohe*).—Much the same symptoms as 3. Whether fatal or otherwise is not mentioned.

5. “*Kukolola*.”—Cough and œdema of chest (? dewlap). Mortality not given.

6. “*Kizengera*.”—Sudden onset. Animal turns round and round and falls. Mortality not mentioned.

7. “*Kalusu*.”—A severe stomatitis, with great swelling of the lips and tongue. The affected animal is unable to eat. Mortality variable.

Remarks.—In 1909 the Commission examined many sick oxen in the Uganda Kingdom, and investigated two outbreaks of cattle disease affecting herds containing over 400 head. Two diseases only were met with—“*amakebe*” amongst calves and trypanosomiasis amongst the cattle. *Trypanosoma pecorum*, *Trypanosoma vivax*, and *Trypanosoma nanum* were the trypanosomes found in the blood of the affected oxen. The diseases, “*Kamenyo*,” “*Kaboa*,” “*Kukolola*,” and “*Kizengera*,” possibly describe different stages in one or other of these two trypanosome diseases of cattle. The disease “*Kalusu*” suggests actinomycosis.

Ankole.

1. “*Amashuyu*” (*Amashu*).—This is the Ankole name for “*Amakebe*.”

2. “*Omurashu*.”—Gradual onset; progressive emaciation; death.

3. “*Habwohi*.”—Sudden onset. Affected animal lies down, or falls, and death follows in a few hours to a few days.

4. “*Tshagama*.”—Like “*Omurashu*.” Mortality not given.

Remarks.—As in the Uganda Kingdom, “*Amakebe*” is prevalent throughout Ankole. The symptoms of diseases 2, 3, and 4 suggest trypanosomiasis,

Toro.

The Toro reports only give the native names of the diseases; no symptoms. The diseases are "Makebe," "Kahora," "Ebite," "Ekihnya," and "Obukigi." The last-named is stated to be caused by the bite of a fly called "Kamgo."

Unyoro.

1. "*Kawola*."—Progressive emaciation, said by natives to be the Sleeping Sickness of cattle. Old oxen invariably die, young stock sometimes recover, but if cows, their calves are cast in the following year after onset.

2. "*Kawawa*."—Effusion of fluid into the chest cavities (pleuræ). The liver and stomach are said to show abnormal changes at death. The disease is very fatal to cattle.

The Nile Province.

1. "*Sadow*."—A dysentery. Occurs in months of June, and is always fatal. Reported from the Nile District.

2. "*Lira*" (?).—No symptoms given. The name used is that given locally to the tsetse fly. Reported from Nimule District.

3. "*Lokkedo*."—Failure of milk is given as the earliest symptom; this is followed by coughing and diarrhœa. If a cow be in calf, miscarriage occurs. Finally, there is marked wasting, with staring coat and running from the eyes, and death. *Post-mortem*—Liver shows enlargement. Attributed by the natives to the game, especially the elephant and buffalo. They say that these animals poison the pasturage by their dung. This is the chief cause of mortality amongst cattle in the Bari District.

4. "*Lobi*."—A gradual wasting disease not accompanied by diarrhœa. Reported from the Bari District. Said to be conveyed from the buffalo to cattle by means of a small buffalo tick.

Remarks.—"Lira" and "Lokkedo" strongly suggest trypanosomiasis. It is interesting to note that the Nile Province would appear to be free from "Amakebe."

Unyoro.

1. "*Kabugo*."—Said by the natives to be caused by the cattle eating grass which has been poisoned by water-buck, giant pig, and bush-buck. There is gradual emaciation with increasing weakness. Staring coat and diarrhœa. Death frequently ensues in two or three days.

2. "*Karungu*" bite (*Bigwa*).—The affected animal becomes weak, lies about, and is unable to stand or walk. There is rigidity of the limbs, and a skin rash. The meat is said to taste bitter and is unsuitable for eating.

3. "*Amakebe*."—Causes many deaths.

4. "*Dwakepunpuru*."—There is vertigo and diarrhœa, followed by death.

5. "*Tchumu*."—Sudden onset. Affected animal falls to the ground and dies in a few hours. This disease is very common and causes many deaths.

Remarks.—The symptoms of the diseases given suggest trypanosomiasis.

Bukedi.

1. "*Angarwa*" (*Angarwe*).—This is the local name for "Amakebe."

2. "*Chumu*" (*Deki*).—Breathlessness, running from the eyes and nose. No cough. Death often follows in 24 hours.

3. "*Fumbi*."—Oedema of the neck and legs. Mortality not given.

4. "*Kitchwamagongo*."—Paralysis of the hindquarters.

5. "*Mugwemge*."—A skin disease with superficial sores all over the body.

6. "*Maiebi*" (*Owala*).—Hair ball in stomach, accompanied by digestive disturbances and coughing.

Remarks.—The names given in brackets are the local Lango dialect names. The other names are in the Banyoro language. Diseases 2, 3, and 4 suggest trypanosomiasis.

Usoga (Busoga).

1. "*Amakebe*."—Described above.

2. "*Nsotoka*."—Described above. No seasonal prevalence.

3. "*Rubi Amiri*."—A disease characterised by great enlargement of the gall-bladder. One diseased animal said to infect a herd. Always fatal. Affects other domestic animals. Occurs in the hot season.

4. "*Kalusu*."—Described above. No seasonal prevalence.

5. "*Naganga*," or "*Jua*."—Hoofs crack and sometimes sepsis and death ensues. Not seasonal.

6. *Redwater*.—Is known, but is extremely uncommon, and has no native name.

7. "*Musuja*."—The word means fever, and so probably a variety of diseases are covered by this name. Attributed to the bites of "*Nkwa*," the native name given to most of the tick in the country.

Elgon District.

1. "*Amakebe*."—Called, locally, "*Angarwe*."

2. "*Sifua*."—Occurs in epidemic form about every five years amongst the Bagishu cattle. It is characterised by joint swellings; legs chiefly affected. Death always ensues.

3. "*Kawawa*."—A disease said to be very prevalent in the vicinity of Mbale. It is attributed to the bite of a tick called by

the same name, “ Kawawa.” It is a chest disease, characterised by great difficulty in breathing.

QUERY 10.

Do the natives attribute the disease to any blood-sucking insect?

This query has been answered above, under Queries 5 and 9.

QUERIES 13 AND 14.

Have you a disease called “ Amakebe ” among calves in your district? Is this disease restricted to certain parts, or does it occur everywhere?

With the exception of the Nile Province, this disease occurs throughout the entire Protectorate. The disease is more prevalent and has its high mortality in the important townships, large missionary stations, and villages on the main routes of cattle traffic. For full details of this disease, reference should be made to the Commission’s paper on this subject, entitled: “ Amakebe: a Disease of Calves in Uganda.”*

It is difficult to come to any definite conclusion from the reports given above of the various cattle diseases in the Uganda Protectorate. The Commission are, however, of the opinion that the two most common and constant cattle diseases in Uganda are “ Amakebe,” chiefly affecting calves and young stock, and trypanosomiasis, affecting the mature animals. There is evidence that at long intervals an epidemic disease occurs of great virulence and causing many deaths, and that this disease resembles rinderpest. Finally, it is not improbable that cases of actinomycosis occur in the Uganda Kingdom and Busoga.

APPENDIX.

The following gentlemen furnished reports on cattle disease, &c., in reply to the Commission’s circular letter, dated June 15, 1909:—

R. D. Anderson, Esq.; D. L. Baines, Esq.; E. Brown, Esq.; Rev. H. Clayton; J. de G. Delmege, Esq.; Rev. T. B. Fletcher; Captain A. D. Fraser, R.A.M.C.; Dr. J. H. Goodliffe; E. B. Haddon, Esq.; C. F. H. Henry, Esq.; F. M. Isemonger, Esq.; Rev. Father Jackson; G. B. Jervoise, Esq.; Rev. A. L. Kitching; F. A. Knowles, Esq., C.M.G.; Rev. Father Lafleur; Rev. — Millar; E. G. Morris, Esq.; M. Moses, Esq.; Dr. H. B. Owen; R. Paske Smith, Esq.; Rev. Father Pelger; Rev. F. Rowling; Rev. H. W. Tegert; Capt. H. M. Tufnell; Dr. R. van Someren; Archdeacon Walker; A. H. Watson, Esq.; Arnold Witlox, Esq.; H. P. Wright, Esq.

* ‘Proceedings’ of the Royal Society, B, Vol. 82, p. 256, 1910, and Reports of the Sleeping Sickness Commission of the Royal Society, No. X., p. 86, 1910.

APPENDIX I.

SUMMARY OF METEOROLOGICAL OBSERVATIONS (UNCORRECTED) CARRIED OUT AT MPUMU, CHAGWE, UGANDA (0° 15' N., 32° 50' E.), FROM 1ST NOVEMBER, 1908, TO 31ST OCTOBER 1909. HEIGHT (APPROXIMATE) ABOVE SEA-LEVEL, 4,300 FEET.

Month.	7 a.m.						2 p.m.				9 p.m.					
	Barometer.	Thermometer attached.	Dry bulb.	Wet bulb.	No. of days rain fell.	Total amount of rain.	Barometer.	Thermometer attached.	Dry bulb.	Wet bulb.	Barometer.	Thermometer attached.	Dry bulb.	Wet bulb.	Maximum thermometer.	Minimum thermometer.
1908.																
November	—	—	70·85*	64·95*	15	9·22	—	—	—	—	—	—	76·50*	67·10*	78·60*	62·00*
December†	25·823	66	65	62	15	5·44	25·774	70	74	65	25·783	68	67	63	80	61
1909.																
January ...	25·795	67	66	62	7	1·57	25·739	73	77	76	25·722	70	68	63	80	61
February	25·789	69	68	64	4	0·20	25·736	75	79	66	25·741	72	70	64	83	63
March ...	25·780	68	67	64	15	5·72	25·654	75	77	67	25·750	71	69	65	80	63
April ...	25·794	66	64	63	24	8·49	25·756	71	74	68	25·788	69	67	65	76	62
May ...	25·815	67	67	64	15	4·98	25·784	71	75	68	25·816	70	67	64	78	62
June ...	25·842	66	64	62	13	2·78	25·812	69	73	67	25·841	69	66	63	76	62
July ...	25·853	66	64	62	14	3·92	25·828	69	72	66	25·841	68	66	62	77	61
August ...	25·838	64	62	59	15	4·92	25·806	69	75	66	25·833	68	65	62	79	60
September	25·834	65	63	62	14	2·06	25·784	69	73	66	25·815	68	66	63	79	60
October ...	25·811	67	65	62	18	7·02	25·762	71	76	67	25·780	70	68	64	81	61
Totals ...					169	56·32										

* These readings were carried out at 9 a.m. and 3 p.m., in the laboratory.

† Barometric readings commenced on December 19, 1908. Meteorological station erected December 1, 1908.
N.B.—The above figures are, with the exception of those given in the two rainfall columns, monthly averages uncorrected.

Observer, JAMES WILSON.

APPENDIX II.

REPORT ON AN OUTBREAK OF DISEASE AMONG CATTLE ON THE NAMUKEKERA ESTATE BELONGING TO THE UGANDA COMPANY, LIMITED.*

INTRODUCTION.

The object of publishing this report is to draw the attention of anyone in Uganda interested in cattle to the subject of their diseases. The diseases of stock in tropical countries are many and various, and, as a rule, are caused by very minute parasites belonging to the animal kingdom, and not to bacteria or fungi, which so frequently cause disease in temperate climates. But, it may be asked, what has a Sleeping Sickness Commission to do with cattle diseases? The answer to that is, that cattle diseases of Uganda are so closely related to Sleeping Sickness in man, that the study of any of them may throw new light on the others.

Sleeping Sickness and many of these diseases of cattle in Uganda are caused by one genus or kind of animal parasite, the trypanosome, an

* Copies of this and the following Reports on cattle disease were circulated to the officials, missionaries, traders, &c., in Uganda.

exceedingly minute creature, about 1/2000 of an inch in length and 1/10000 in breadth. It lives and multiplies in the blood, and it is only by means of some biting insect that it can be conveyed from one animal to another. Sleeping Sickness, for example, is caused by the *Trypanosoma gambiense*, and it is carried from the sick to the healthy by the *Glossina palpalis*, one of the tsetse flies. Wherever that fly is there you may have Sleeping Sickness; wherever there is no *Glossina palpalis* there can be no Sleeping Sickness. The distribution of the fly is peculiar, as it is only found at the edge of clear water, such as the Lake, the Nile, or the clear running streams in Kavirondo. It is also only found where there is forest growing up to the edge of the water, so as to afford it shade. So that, in the whole of Uganda, this fly is only seen along the shore of the Lake, the Nile and Lake Albert, and only where forest fringes the banks.

It therefore results that, with this knowledge, it is perfectly simple to avoid this disease. If a man does not expose himself to the bite of this particular fly he cannot get Sleeping Sickness. And in the same way, with this knowledge, it is a simple matter to stamp out this disease, all that is required being to clear the places where this fly is found of their human population, and the epidemic at once ceases. No fresh cases can arise. This was discovered and described by the Commission in 1903.

Now, in the same way, if the cause of the cattle diseases is known and the insect which carries the infection is discovered, then probably it will also be simple to prevent or stamp them out. This work is important. From want of this knowledge thousands of cattle in Uganda die annually, and from want of this knowledge fresh cases may be allowed to slip into the country which will sweep the pastures clean.

This report, then, is not only written to draw attention to the cattle diseases, but also to enlist the aid and advice of everyone in the country interested in stock. The Commission sent round a circular on the 12th December, 1908, asking among other things for information in regard to these cattle diseases, but with the exception of Sir Apolo Kagwa, K.C.M.G., the Katikiro, Mr. Russell, the Director of Transport, Mr. Tyler, the General Manager of the Uganda Co., Ltd., and one or two others, no notice has been taken of it. That these diseases are important in Uganda is shown by the Prime Minister's letter in *Uganda Notes*, March, 1909, in which he says that there are three diseases among his cattle:—"Kimpupuli," "Lukampa" or "Lukulu," and "Amakebe." He writes that these diseases destroyed very many of his cattle during the year 1908. He had 1,396 cows which had 2,021 calves, and of these calves 709 died, or 35 per cent. Further, of cows, bulls and full-grown bullocks, there have died 351. That makes a total of 1,060 deaths in one year. Sir Apolo says this percentage of deaths is monstrously large. During the same year the Uganda Company lost about 20 per cent. of the cattle working on their estate, and 50 per cent. of their transport oxen, and the Transport Department, in Entebbe, 156 animals between June and November. If 1,060 deaths take place in one herd, what must the number be for the whole of Uganda? In this way many thousands of pounds sterling are lost yearly by the disease, and yet nothing is done to prevent the waste.

Description of Uganda Company's Estate at Namukekera.

This estate is situated in Bulemezi, in Uganda, Lat. 0°45' N., Long. 32°30' E., and lies at an elevation of about 4,000 feet above sea level. It is within four miles of the Maanja River, and consists of open undulating grass country, which in many parts is swampy in the rainy season. Total area 1,583 acres. Here and there are small clumps of mimosa, euphorbia and other trees. The antelope found in this part of the country are principally Thomas' kob, water-buck, bush-buck, reed-buck, duiker, and a few zebra. The estate is surrounded on two sides by hills, which are covered with low scrub. The cattle are watered during the dry season at the Maanja River, four miles away, and on the estate itself during the rainy season. The cattle on the estate number 234, and are employed for farming purposes. They are bought locally, at Kampala, or at Kisumu in Kavirondo.

The following table gives a list of the sick cattle, with the name of the particular trypanosome disease they are suffering from, and, as far as possible, the district the oxen came from:—

Experiment No.	No. of Ox.	Date.	Age.	Breed.	Where bought.	Route taken to Namukekera.	How long on estate.	How employed.	When taken sick.	Present condition.	Nature of disease.
<i>Trypanosoma vivax.</i>											
493	14	2.2.09	5	Kavirondo	Kisumu ...	Old caravan road	1½ months	Plough ...	1.2.09	Dead	Wasting.
496	1	2.2.09	3-4	Ankole and Uganda.	Mr. Leakey Kibinji.	Local path	10 "	No work ...	1.1.09	"	"
450	—	2.2.09	2	Kavirondo	Kisumu ...	Old caravan road	—	—	1.1.09	"	"
<i>Trypanosoma pecorum.</i>											
495	9	2.2.09	4-5	Uganda ...	Locally	Kampala one week.	18 months	Plough ...	1.9.09	Emaciated	Wasting.
502	99	5.2.09	8-10	Kavirondo	Kisumu ?	Old caravan road	15 "	" ?	?	Thin, deplap fluid	"
503	100 B	5.2.09	?	Kavirondo	?	A few months	?	Transport	?	Thin and ill	Wasting.
504	105	5.2.09	—	Uganda ?...	Kampala ...	Porters road to Massindi.	9 months	Plough ...	5.2.09	Heathy looking ...	"
505	87	5.2.09	4-5	Uganda ?...	Kampala ...	Old caravan road	?	?	?	"	"
534	171	8.2.09	—	Kavirondo	Kisumu ?	Old caravan road	?	Transport	?	Killed	"
536	4	12.2.09	8-9	Ankole ...	?	Kampala. Transport.	?	?	1.8.08	"	"
541	42	11.2.09	—	?	?	?	?	?	13.2.09	?	Rapid.
550	Cow	15.2.09	Old	?	?	?	8 months	?	15.2.09	Thin	Wasting.
576	—	8.2.09	—	?	?	?	?	?	?	?	?
<i>Trypanosoma ingens.</i>											
533	100 W	9.2.09	2	Uganda ...	Kayiwagobi	Along native path	9 months	Nil	?	Healthy	?
494	48	3.2.09	—	Kavirondo	Kisumu ...	Old caravan road	1 "	?	?	"	?

On the 29th January, 1909, at the suggestion and invitation of Mr. E. H. Tyler, the General Manager of the Uganda Co., Ltd., Captains H. R. Bateman, R.A.M.C., and F. P. Mackie, I.M.S., members of the Sleeping Sickness Commission, proceeded to Namukekera to study the diseases of the cattle on the estate. They arrived there on the 31st January, and were most hospitably received by Mr. Lea-Wilson, the Assistant Agriculturist, who gave them every possible assistance. These officers remained on the estate until the 16th February, and during that time examined all the Company's oxen. Of these nine were reported as being sick. Three of these nine were found to have trypanosomes in their blood. Among the apparently healthy, twelve others were also found to be suffering from trypanosome disease.

It must be mentioned at the outset that the parasites causing these cattle diseases are named *Trypanosoma vivax*, *Trypanosoma pecorum*, &c., because those causing the Uganda diseases approximate in type to these named trypanosomes. The Commission cannot at present assert that they are identical. Work is being done to settle this question. But in this report it will be taken for granted that in Uganda we are dealing with *Trypanosoma vivax* and the others.

Trypanosoma vivax is the name of a trypanosome which causes a common fatal disease amongst cattle in Uganda. It is carried from sick to healthy cattle by the same species of tsetse fly—*Glossina palpalis*—which spreads Sleeping Sickness. Antelope inhabiting the lake-shore have been found to harbour this trypanosome in their blood. It is, therefore, possible that the flies originally got the infection from wild animals, and subsequently passed the disease to domestic cattle and goats, although nowadays the disease is probably conveyed from sick to healthy stock when they are grazing or watering at fly-infested lakes or rivers.

Trypanosoma pecorum.—This is a disease of cattle very widely spread over Central Africa. It occurs in Senegal and the Congo territories on the West, and has been reported from Zanzibar, Mozambique, the mouth of the Zambesi, and Zululand, on the East. Its carrier is not exactly known. Many observers blame the *Glossina palpalis*, the same tsetse fly which carries the infection of Sleeping Sickness. Mr. Montgomery, who has spent the last two years in Nyasaland, writes that there is damning evidence against the *Stomoxys*. If the *Stomoxys* carries this infection then it will indeed be widespread, as this fly is found almost everywhere. The *Stomoxys* is the small black fly, exactly like the common house-fly, which is seen so often on cattle. The Commission believe that by finding out the history of a number of cattle affected by this disease—where they come from, and where they have been—some light might be thrown on the subject of the carrier. For example, if a number of cattle are found to have *Trypanosoma pecorum* in their blood which have never left a particular district, then the carrier must be found there. If no *Glossina palpalis* are found in that district, then it cannot be this particular fly which carries it. If, on the other hand, the disease was only found among cattle which had been used as transport animals to some point on the Lake, such as Luzira, where *Glossina palpalis* abound, then this would implicate the tsetse fly. If the list of the Namukekera oxen is examined, it will be seen that the information given is not sufficient to enable an opinion to be given. There are ten oxen affected, but the origin of only four can be traced. Two were brought in Kisumu and two locally. This brings out the necessity of stock owners keeping a stricter account of the origin of their cattle if they wish to help this Commission in its search for the carrier of this disease. Every ox should be numbered or marked in some way and entered in a book with its history.

Trypanosoma ingens.—This is a little-known and probably harmless disease of cattle.

These, then, are the principal cattle diseases found in the Namukekera herd. *Trypanosoma vivax* and *Trypanosoma pecorum* are both fatal diseases for cattle, and, up to the present, there has been found no means of curing them. What the Commission wishes to find out is the carrier of *Trypanosoma pecorum*, and this report has been written to draw attention to the matter.

In regard to the three cattle diseases mentioned by Sir Apolo Kagwa in his letter, there can be little doubt that the first two are trypanosome diseases, while the third, "Amakebe," is probably caused by another kind of parasite altogether—a piroplasma. These piroplasma diseases are found all over the world, and are conveyed from one animal to another by means of ticks.

MPUMU, CHAGWE,
27th March, 1909.

APPENDIX III.

REPORT ON AN OUTBREAK OF DISEASE AMONG CATTLE ON THE ESTATE OF A. B. WALSH, ESQ., AT KABULA MULIRO.

By Captain F. P. MACKIE, I.M.S.

News having reached Mpumu that an outbreak of disease had occurred amongst the cattle on Mr. Walsh's estate, the writer and Mr. E. Hutchins, M.R.C.V.S., the Veterinary Officer to the Protectorate, were despatched to investigate the epidemic. They arrived at Kabula Muliro on July 20, 1909, and were most hospitably entertained during their stay by Mr. and Mrs. Walsh.

The exact conditions under which the outbreak commenced are somewhat indefinite, as Mr. and Mrs. Walsh were away, but it began suddenly, and the cattle began to die during the second week in June, and by the 16th June thirteen animals had died, seven of which were milking cows with calves at foot. Some others were sick, and of these five died after Mr. Walsh returned. In addition to these losses, the following cattle, the property of the Reverend Mr. Chadwick, also died: two cows, three yearling heifers and a bullock. These died between June 14th and July 1st.

It appears, therefore, that between the middle of June and the middle of July, twenty-four animals had sickened and died. There were, in addition, sixteen sick animals which had been isolated from the healthy herd by Mr. Walsh, and of these ten were subsequently found to be infected and were slaughtered. The total mortality up to the time of writing is, therefore, thirty-four head.

The symptoms which the animals presented during the course of the disease were, briefly, as follows:—The animal is noted to be off its feed, and stands listlessly with its head hanging down. Its eyes are dull and half closed. The coat is staring. There is no discharge from the nose or eyes, but some animals suffer from a foetid watery diarrhoea. The mucous membranes show marked pallor, indicating extensive blood destruction, and the progressive weakness is a further indication of the same process. The temperature is often raised to 104° F. or 105° F. (the normal temperature of cattle lies between 101° F. and 103° F.), and the animal is seen to shiver in cool or rainy weather. After a few days' illness, during which time the animal feeds badly and gets progressively emaciated, it goes down in the kraal and is unable to rise. Latterly it lies on its side, with its head drawn backwards, and some stiffness and quivering in the limbs. Stinking diarrhoea is common during this stage, and the animal dies in a day or two apparently from exhaustion. The temperature during the last few days of life is normal or sub-normal.

The appearance of the organs after death is not characteristic of the disease, but its features are shared in common with other conditions associated with severe anæmia. The most marked feature, which would strike those unfamiliar with *post-mortem* examinations of cattle, is the degeneration of the fat around the base of the heart and the kidneys. The fat in such parts, instead of being white or yellow and opaque, becomes degenerated into a transparent jelly-like material. The spleen is often larger than normal, but the important organs of the body do not show marked change. The small and large intestines show evidence of inflammation, which explains the occurrence of diarrhoea in some of the cases.

The microscopical examination of the blood of these cattle revealed the following facts. A small parasite, known as a trypanosome and probably identical with the variety called *pecorum*, was found in eight of the sixteen suspected animals. Another trypanosome, probably identical with that previously described by the Commission and called by them *Trypanosoma ingens*, was found in two of the suspected herd. The remaining six animals which had been isolated on account of sickness did not show trypanosomes, even after repeated examinations.

The remainder of the cattle on Mr. Walsh's estate were then branded and examined, a total of 189 adults and 80 calves. Out of these 269 animals only three were found to be infected, one with *Trypanosoma ingens* and two with *Trypanosoma pecorum*. All the animals which were shown to harbour *Trypanosoma pecorum* were destroyed at Mr. Walsh's request. The three animals whose blood contained *Trypanosoma ingens* were allowed to return to the healthy herd, as the parasite is believed to be harmless.

It seems probable that the cattle get *Trypanosoma ingens* from the wild game in the locality, as it has been found by the Commission to exist in the blood of the bush-buck.

Having outlined the main features of this unfortunate epidemic, there remains to be considered how the infection arose and how it was disseminated. This is the more difficult, as those responsible for the investigation did not arrive on the spot until the epidemic was over, and therefore, the conditions which obtained at the time of the outbreak are largely conjectural. It appears from Mr. Walsh's statement that there has been no case of any similar disease amongst his cattle during the three years he has been at Kabula Muliro. The cattle are exceptionally well housed and tended, and with the exception of the sick herd, were in remarkably good condition.

LIST OF SICK CATTLE ON MR. WALSH'S ESTATE AT KABULA MULIRO WITH TRYPANOSOMES IN THEIR BLOOD.

Experiment No.	Date.	Age. Years.	Breed.	Whence obtained.	Length of time on estate.	Duration of illness.	Remarks.
<i>Trypanosoma pecorum.</i>							
2	1909. July 21	2	Half-bred Ankole heifer.	Home bred	2 years	5 weeks	Killed.
3	"	3	Half-bred Ankole cow.	Mitiana	"	"	"
8	"	4	"	Gomba	3 years	2 weeks	"
9	"	2½	"	"	"	3 "	"
10	"	5	"	"	"	2 "	"
11	"	10 months	Bull calf	Home-bred	10 months	4-5 weeks	"
12	"	3½	Half-bred Ankole cow.	Gomba	3 years	4 weeks	"
16	"	5	Half-bred Busoga cow.	"	"	2 "	"
106	July 27	18 months	Bull calf	Home bred	18 months	Not known	"
169	"	"	Heifer	"	"	"	"
<i>Trypanosoma ingens.</i>							
1	July 21	3	Half-bred Busoga cow.	Mitiana	2 years	5 weeks	Returned to healthy herd.
7	"	2½	Ankole cow	Home-bred	2½ years	2 "	"
151	July 27	20 months	Bull calf	"	20 months	Not known	"

In looking over the accompanying table it will be seen that most of the animals which died had either been bred on the estate or had been on the estate for two or three years. It seems certain, then, that the origin of the epidemic was due to some local and more recent change of conditions, but what these conditions were must, unfortunately, be left undetermined.

Three possible sources of infection may be considered—(1.) From the passage up and down the road of cattle on transport work. (2.) From the occurrence of disease in local native herds. (3.) From the wild game in the vicinity.

It appears that oxen have been going up and down the road between Kampala and Hoima for many months, and it is known that some of these were infected with *Trypanosoma pecorum*. It is possible that they were the source of infection at some past time. A batch of these animals was examined by the investigators above mentioned, and two out of twenty were found to be infected with *Trypanosoma pecorum*. This batch of oxen arrived at Kabula Muliro about ten days to a fortnight before the outbreak of the epidemic amongst Mr. Walsh's herd, but the Commission are of opinion that this interval is far too short to permit of the two events being regarded in the light of cause and effect. It is more probable that the Kabula Muliro epidemic owed its origin to the passage of transport cattle at some earlier date.

The second possibility is, that the epidemic was caused by the presence of infected native herds. The only evidence obtained was against that hypothesis, for though six or seven native herds were inspected, they appeared to be free of disease, though it is true microscopical examinations were not made.

It is possible that the infection may exist in the wild game, and this has been abundantly proved for at least one trypanosome, viz., *Trypanosoma brucei*, which, existing as a harmless parasite in wild game, causes the virulent disease known as nagana when carried to domestic animals by the tsetse fly, *Glossina morsitans*. It is possible that in the same way, the wild game in Uganda may constitute "reservoirs" of *Trypanosoma pecorum* infection, only needing the appearance of a certain biting fly to start an epidemic in cattle. Reference has already been made to the presence of *Trypanosoma ingens* in the blood of cattle, and in that of the bush-buck which feed over the same ground. There remains one problem to be faced, and that the most important of all—that is the presence of biting flies.

In order to place the matter in a clear light, one can draw an analogy between the outbreak of an epidemic and the explosion of a cartridge. The presence of healthy and infected cattle together is not by itself an *actual* source of danger but only a *potential* one, just as the association of the powder and the bullet in a cartridge. The factor which, given the other two, determines the outbreak of an epidemic or an explosion is, in the one case the appropriate biting fly and in the other the percussion cap. The fly is the determining factor in the explosion of a trypanosome epidemic such as the one under consideration, and in its absence infected and non-infected animals may be herded together with impunity.

The matter of prime importance was to ascertain the varieties of biting fly in and around Kabula Muliro. A great deal of attention was paid to this point, and as a result, the following well-known blood-suckers were found to exist in some numbers.

Hæmatopota; *Tabanus* (native name, "Dumanbogo"); *Chrysops*.

All attempts to prove the presence of the true "Kivu"—the tsetse fly—failed, though it is possible if more extended observations could be made some might be discovered.

It is for the assistance and co-operation of others in this matter that the Commission have pleaded in the past and now appeal again. It may be stated definitely that until some accurate idea of the distribution of biting flies in Uganda is obtained, attempts at limiting or stamping out these epidemics will be futile. It is imperative that all those who are in any way interested in the presence of cattle in Uganda, or in opening up the country for transport, or in stock raising, should in duty bound do all in their power to find out the presence of biting flies in their own neighbourhood, by collecting specimens of these and forwarding them to Mpumu for identification and classification. It was chiefly by this means that nagana was stamped out in South Africa, and at least some of the credit for this is due to the public spirit of those concerned.

Note by the Director of the Sleeping Sickness Commission.

The above account of this epidemic, written by Captain Mackie, I.M.S., may be summarised as follows:—

1. In the short space of one month twenty-four cattle died on Mr. Walsh's estate.

2. After the arrival of Captain Mackie and Mr. Hutchins, there were ten more deaths, so that in the course of two months, thirty-four cattle died out of a herd of about 300, and the epidemic came to an end.

3. In the blood of these sick oxen a trypanosome of the *pecorum* type was found by Dr. Sells, Captain Mackie, and Mr. Hutchins. It is to be regretted that the blood of all the animals was not examined to make sure that they all contained this parasite; but there is no evidence that any of the twenty-four oxen which died between the middle of June and the middle of July were so examined. Nevertheless, we must conclude that all these animals died of *Trypanosoma pecorum* disease.

4. *Trypanosoma pecorum* is widespread in tropical Africa, extending from about 20° N. lat., to 20° S. lat. It is common all over the French and Belgian Congo, the Egyptian Soudan, Rhodesia, and Nyasaland. It is, therefore, probably found all over Uganda where the conditions are favourable. In my opinion, many herds of cattle in Uganda, if carefully examined, would show a few with trypanosomes in their blood. This Commission have found it in cattle at Kabula Muliro, Namukeyera, and Mabira, and also in the Government transport cattle at Entebbe and Kampala.

5. Of the course and duration of the disease in cattle caused by *Trypanosoma pecorum*, little, unfortunately, is known. At Mpumu only eleven oxen have come under observation. Nine were brought here sick, and two were infected by the injection of blood containing *Trypanosoma pecorum*. Of the nine, six are dead and three alive. The six died 2, 4, 3, 1, 9 and 34 days after arriving here. The three have lived 248, 198 and 184 days respectively. In regard to the two which were artificially infected, one died after 46 days, and the other is still alive after 172 days. This will show how very irregular, as a rule, the duration of this disease is. It must also be borne in mind that the length of the disease in cattle will vary according to the work they have to do. These trypanosome diseases attack the blood, causing anæmia, and, consequently, weakening of the heart. If, therefore, an animal is used in a team of oxen and has to pull a heavy waggon over Uganda roads, naturally it will soon fall out and die. On the other hand, if it has no work to do, then the disease will probably take months or years to kill. Mr. Walsh's cattle had little to do except graze, so that one would have thought the duration of the disease would have been long.

6. The only conclusion I can come to is that the thirty-four oxen were infected about the same time—and this might possibly have been about the beginning of May or a little earlier—and that the disease ran an acute and rapid course, so that all the infected animals succumbed within three and a-half months of receiving the infection. It may be noted that a few of the animals might have survived for a longer period had they not been slaughtered as a preventive measure.

7. There is no evidence available to point to the particular biting insect which caused this outbreak. The Commission have proved that the tsetse flies can carry this disease, but there does not seem to be any of these flies in this district. It is possible that the *Tabanidæ* and *Hæmatopota* also carry it, but this has never been proved by experiments, or observed under natural conditions.

8. In regard to the means of preventing such an outbreak in the future, I would say that it would be well to keep the herd as much as possible from contact with animals of suspicious or doubtful character—such as the transport animals which pass up and down the Hoima road. This would be best done by fencing, a course which may not be practicable under the present conditions of the country. Then a watering-place should be chosen on the river as far as possible from those used by other herds. Lastly, a careful watch should be kept on

the herd in order to detect, as soon as possible, any animal which may have become infected with trypanosomes. If such an animal is found it should be at once slaughtered, as it can be of little use either for work or milling purposes, and it acts as a reservoir of the virus. It might be well to keep a microscope on the premises in order that the diagnosis could be made with the least possible delay.

MPUMU, CHAGWE.

20th August, 1909.

APPENDIX IV.

MEMORANDUM ISSUED BY THE DIRECTOR OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY, UGANDA, 1908-1910.

1. This Commission has been sent out to Uganda by the Colonial Office to continue the investigations initiated in 1903.

2. In 1903 the Commission discovered that Sleeping Sickness is caused by the entrance into the blood of a minute parasite, the *Trypanosoma gambiense*, and that this trypanosome is conveyed from the sick to the healthy by means of a particular fly, the *Glossina palpalis*, and by it alone.

3. The distribution of this fly was then worked out and found to coincide exactly with the distribution of Sleeping Sickness, a strong proof that this fly is the only carrier.

4. The distribution of this species of tsetse fly was found to be a peculiar one. It was only found on the shore of the Lake, or on the banks of open rivers, such as the Nile, where there is plenty of forest shade. This rendered it easy for intelligent persons to escape this absolutely fatal disease, as all that was required was to keep out of the fly area, or, if it was necessary to pass through it, to take precautions against being bitten by the fly. It also enabled preventive and stamping out measures to be organised on a rational basis.

5. The last Sleeping Sickness Commission continued to work until March, 1906, when one of the members, the late Lieutenant Forbes Tulloch, R.A.M.C., most unfortunately contracted the disease, and this sad event brought the work of the Commission to an abrupt close.

6. It had been the intention of the Royal Society to keep the scientific Commission constantly employed, by sending out fresh workers from time to time, until all the main diseases, both of men and animals, had been described, and their distribution mapped out. It was truly said to the Commission the other day by Lord Delamere, that it is only by science that these Colonies can progress. In the last report of the Government Veterinary Bacteriologist, Transvaal, it is stated that the sum of money voted by the Legislative Assembly to his department alone for the current expenditure of the year was £30,934. In the Transvaal this money is considered well spent.

7. If the programme of the Royal Society had been carried out, no doubt much good would have accrued to the Protectorate. Examples illustrating this could be multiplied: in 1903, when Mr. Grant brought some 1,500 cattle from Mount Elgon to Jinja, he rested them in a valley swarming with *Glossina morsitans*, and as the result lost them all from nagana. A little knowledge of the distribution of this deadly fly would have prevented this loss. Again, at the present time, the Uganda Government are losing numbers of their oxen from an unknown disease. If the cause of it could be discovered, and its mode of spread traced, this mortality among the stock might be prevented. Thanks to the courtesy of the Principal Medical Officer, Dr. A. D. P. Hodges, C.M.G., who thinks the disease may be caused by *Trypanosoma pecorum*, and the Transport Officer, Mr. Russell, the Commission are having the opportunity of seeing examples of this disease. Yet again, the Commission are informed that from 40 per cent. to 60 per cent. of calves die of a disease called "Amakebe." This seems a large mortality, and something might perhaps be done to lessen it.

The Commission, thanks to the kindness of the Katikiro and the Sekibobo, will also have an opportunity of seeing this disease.

8. During the last few years the knowledge of the distribution of the *Glossina palpalis* and of Sleeping Sickness in Uganda has been much advanced, due chiefly to the energy of the Principal Medical Officer, Dr. Hodges, and Dr. A. G. Bagshawe, at present Director of the Sleeping Sickness Bureau in London. It is gratifying to know that nothing has been discovered to shake the conclusions arrived at by the Commission in 1903.

9. The chief work of the present Commission is to continue the study of Sleeping Sickness in the Protectorate, and for this reason this memorandum is being sent to officials, missionaries and others, to invoke their aid and criticism. At the same time the Commission is ready to undertake the investigation of any disease, whether of men or animals, which may be brought to their attention.

10. Since 1903 evidence has been brought forward, especially from the Congo, to the effect that Sleeping Sickness is spread not only through the agency of *Glossina palpalis*, but also by other species of tsetse flies, other biting flies, and even by fleas and such-like insects. Now, if anyone can bring forward any proof of cases of Sleeping Sickness arising in districts free from *Glossina palpalis*, the Commission would be greatly assisted. The theory held by the Commission is that the tsetse fly, *Glossina palpalis*, is the only carrier. Naturally, it is most important to know if other biting insects can also act as carrier.

11. Again, His Excellency the late Professor R. Koch of Berlin has advanced the theory that close contact, as, for example, when an infected man returns from the Sleeping Sickness area to his wife and children living in a fly-free area, is also a means of spreading the disease. The importance of this cannot be exaggerated. The Commission hold that this never occurs, but invite information and criticism.

12. Another important point which is engaging the attention of the Commission is whether it can be shown that any other animal, except man, is capable of acting as a reservoir of the virus. The importance of this is self-evident. For example, the monkey is very susceptible to this disease, and dies in a few months after infection. If the monkeys along the Lake-shore are infected, then the removal of the Lake-shore population will not render that area healthy, as the *Glossina palpalis* will be able to get the virus from the monkey. This also holds good of the hippopotamus. The Commission do not believe that this occurs, but wish to examine the blood of as many monkeys and hippopotami as possible. The Commission would like, therefore, to have sent as many living monkeys from the Lake-shore as possible, especially from places where Sleeping Sickness is prevalent. Also, is there any evidence that monkeys have been dying of Sleeping Sickness? It is reported that the monkeys have disappeared from Buvuma. Is this true? In the event of a hippopotamus being killed on land or shallow water, the heart full of blood might be sent post haste to the Mpumu laboratory. The blood vessels at the base of the heart would be required to be tied tightly, and the heart wrapped in damp grass or banana leaves to keep it cool.

13. In tropical countries the chief disseminators of disease are blood-sucking insects. For example, malaria is carried by certain mosquitoes, Sleeping Sickness by a tsetse fly, the *Glossina palpalis*, tick fever by a tick, horse sickness probably by a mosquito, East Coast fever and redwater by cattle ticks, jaundice in dogs by a tick, and so on. It is, therefore, very important that the different species of these biting insects should be known and their distribution mapped out. Their habits (such as feeding by day or night), their habitat (such as living in houses, villages, woods or marshes), &c., should also be studied. The Commission would, therefore, be greatly obliged if anyone interested in these matters would send in specimens of biting flies, mosquitoes, ticks, fleas, &c. All that is necessary is to kill them, enclose them in a bottle, a gourd, or a match-box, and address to the Sleeping Sickness Laboratory, Mpumu, with an enclosure giving the native name, place of capture, &c. All such specimens will be fully acknowledged in the official reports of the Commission.

APPENDIX V.

REPORT ON THE JINJA PENINSULA AS TO THE INFECTIVITY OF THE TSETSE FLIES FOUND THERE.

By Captain A. E. HAMERTON, D.S.O., R.A.M.C.

A certain part of the Lake-shore near Jinja was reported to be infested with tsetse flies, and to be frequented by people suffering from Sleeping Sickness. A member of the Commission—the writer—was therefore despatched on February 18, 1909, to investigate the prevalence of *Glossina palpalis* in this place, and to conduct an experiment of feeding tsetse flies on a healthy monkey, in order to ascertain if the tsetse flies found there were infective.

Jinja was reached on February 20, 1909, and on the 21st the locality in question was examined. It may be described as a small peninsula running out into the Lake, about three-quarters of a mile to the east of Jinja station, and terminating in a conical hill, on the top of which the principal chief of Usoga, known as the Semei Kakunguru, has his residence. This peninsula is about one and a-half miles long by half a mile in breadth. A broad public path, leading to the chief's house, traverses nearly the whole length of the peninsula. On each side of this path there are banana plantations and huts. A low swampy shore, overgrown with forest and papyrus, intervenes between these shambas and the water's edge. Towards the end of the peninsula there are no shambas and the forest comes right up to the path.

A number of labourers were employed on this peninsula in building houses for the Kakunguru. Some of these men were seen at work on a house at the foot of the hill and to the right of the public path. They were working amidst the trees and bush of the forest, and within a few hundred yards of the water.

Tsetse flies were very numerous about the building, and had ample opportunity for biting many individuals. Provided that some of the workmen had trypanosomes in their blood: the ideal circumstances required for the distribution of the parasite from the sick to the healthy, were here present. Tsetse flies were observed in twos and threes throughout the whole length of the path running down the centre of the peninsula, along which many people were passing to and fro or loitering in the shade of the trees.

Grey monkeys were common in the belt of the forest. Two of them were shot in a part of the peninsula infested with enormous numbers of *Glossina palpalis*. One of the monkeys was disabled, and whilst lying on the ground it was watched for about half-an-hour to see if the flies would feed upon it. No flies settled on the wounded monkey, although they were swarming on and around the person of the observer. A *post-mortem* examination was made on the monkey in order to obtain preparations of its heart's blood, liver, and spleen. Microscopical examination of these preparations showed abundant malarial infection in one monkey. No trypanosomes, filaria or spirilla were found in the blood of either of them. Another monkey was shot near the same place. It bled profusely where it fell, but no tsetse flies were attracted by the blood freshly shed upon the ground, although other species of flies fed readily upon it.

It may be noted that a mosquito veil and gauntlets cannot be considered adequate protection against the bites of tsetse flies in such a place as this. The flies swarm around the observer and bite freely through those parts of clothing which are tightly stretched over the subjacent skin, as about the knees and buttocks when stooping.

Between the fly-infested peninsula and the station of Jinja there are three water holes whence the natives draw water. These water holes were visited during the heat of the day. A few tsetse flies were found around each of them. All bush and forest has been cleared from the neighbourhood of these water holes. They are surrounded by rank grass, and in one case, by papyrus. These water holes were visited again at 5 o'clock in the evening, when large numbers of people were drawing water. No tsetse flies were observed about the water holes at this hour.

The fly-feeding experiment having been started, Dr Goodliffe kindly undertook to see that it was carried out.

Monkey, Experiment 568, was returned to Mpumu on February 28 by Dr. Goodliffe, who stated in his letter that the Administration had not

removed the people from the peninsula, but that they intended clearing the place of forest. Between February 21 and February 26 Dr. Goodliffe had placed 239 flies to feed on the experimental monkey; the number which actually fed was not estimated. The blood of this monkey was examined at Mpumu on March 4, and it was found to be heavily infected with *Trypanosoma gambiense*. If the incubation period is presumed to be seven days from the exposure to the appearance of the trypanosomes in the blood, it will be seen that this monkey was infected on the third day, by which time 178 flies had been fed upon it.

CONCLUSION.

That the tsetse flies on the Jinja peninsula were infected with Sleeping Sickness in February, 1909.

APPENDIX VI.

AN EXPERIMENT TO ASCERTAIN AT WHAT POINT ON THE ROAD BETWEEN KAVIRONDO AND UGANDA, CATTLE BECOME INFECTED WITH TRYPANOSOMES. AN ACCOUNT OF A JOURNEY FROM KISUMU TO JINJA.

By Captain A. E. HAMERTON, D.S.O., R.A.M.C.

In 1909 the Commission was informed that there was a fly-belt in Usoga, intercepting the old caravan track between British East Africa and Uganda somewhere between Mumia's and Jinja, and that cattle fell sick and died soon after passing through this country. Since Sleeping Sickness and the famine had nearly depopulated Usoga, the roads were abandoned, and no reliable information was obtainable as to what was the species of fly, its distribution, or even if the said fly-belt still existed.

Through the courtesy of Mr. E. H. Tyler, the Manager of the Uganda Company, Ltd., a herd of fifty oxen was placed at the disposal of the Commission for the purpose of making the experiment of driving them through the supposed fly area.

Permission having been obtained from the Administration to bring these cattle overland from British East Africa into Uganda, a member of the Commission—the writer—proceeded, in April, 1909, to Maseno Estate, in Kavirondo, British East Africa. At Maseno, the blood of all the cattle was microscopically examined for trypanosomiasis, with negative results. Mr. Thomas, the Government Veterinary Officer, also examined the cattle and pronounced them all healthy, and granted the necessary permit for them to be driven overland to Uganda. These oxen were natives of Kisumu Province, and had never been out of the Province. Our object was to march with these trypanosome-free cattle day by day along the road to Uganda, to observe the incidence and prevalence of the different species of blood-sucking flies *en route*, to define their limits and, if possible, to identify the species responsible for infecting the cattle with any trypanosome diseases they might subsequently develop.

We left Maseno on April 30 and drove the cattle for about eight miles over undulating country covered with short grass. The Kavirondo people of this district are rich in cattle, and said that they had no disease amongst their herds. On reaching the Yala river we found it in flood. The cattle, however, were successfully swum across the stream, the porters and baggage making the passage in a "dug-out" canoe. The Yala river at this rainy season is a swollen torrent, flowing in a shallow valley, with swampy meadows extending from 100 to 200 yards on either side. The banks of the river were examined for a mile in each direction of its course; no tsetse flies, *Tabanus* or *Hæmatopota* were seen. A few transport cattle and donkeys were grazing in the meadows; their owners, Indian traders, told us that they had no disease amongst their donkeys or cattle. Mr. McNaughton, of the Public Works Department, who was building a bridge over the river, said that there were no tsetse flies in the neighbourhood, and no cattle sickness, but lower down the Yala, where the river loses itself in a swamp near the Lake, tsetse flies were numerous. The dirty kraals, where the cattle were penned after grazing, were infested with swarms of *Stomoxys*.

We reached Mumia's on the evening of May 1, having travelled some twenty-four miles from Maseno. The country traversed was open undulating grass land, swampy and water-logged in parts. Numerous herds of healthy-looking cattle and transport donkeys were passed along the road. A large tributary of the Nzoia river was crossed and its banks examined for tsetse flies. No biting flies were observed on this river, the banks of which are devoid of forest or bush. The Hon. K. R. Dundas, District Commissioner at Mumia's, stated that there was no cattle disease in the vicinity of Mumia's, but that cattle coming into Mumia's from Kakamega and Kebra's usually die within three months from an unknown disease. Kakamega lies twenty-five miles east by south of Mumia's. No fly reported on the road, which runs through open country all the way. Mr. Dundas kindly gave the following information about fly-belts which he met with, or which were reported to him, during a recent journey between Lake Baringo and Mount Elgon. Tsetse flies (species unknown) were met with all along the Kerio river, running in the Legeo valley, between Mount Elgon and Lake Baringo. This country is covered with mimosa bush, with occasional areas of open grass land. Buffalo, rhinoceros, and antelope are numerous in the valley, and cattle grazing in the district are said to be healthy. During the passage of this fly-belt, donkeys were bitten by the tsetse flies, and sickened and died within eight days. The Hon. K. R. Dundas further stated that he encountered another fly-belt along the course of the Wei river, an affluent of the Turkwell, flowing to the N.E. of Mount Elgon, and that tsetse flies were reported all along the banks of the Turkwell river to the north of Mount Elgon. In Lorogi, between the eastern boundary of Baringo district and the Rift valley, Mr. Dundas met with a tsetse-fly (species unknown) belt. His donkeys died within eight days after meeting with this fly-belt. His camels were apparently not affected until a month afterwards, when they sickened and died suddenly. It is said that sheep and goats can be passed through this belt with slight loss. The Lorogi district is inhabited by elephant, buffalo, and rhinoceros, which are said to live within the fly-belt. Antelope and zebra are common outside the fly-belt. Permanent water is found in pools throughout Lorogi. It is said that the Sondai valley, to the south of Lake Baringo, is infested with tsetse flies. In the Mumia's district Sleeping Sickness is prevalent in the following localities:—

1. Alego sub-district, thirty miles south of Mumia's, around Lake Gangu.
2. Wanyala, near the junction of the Sio river with Lake Victoria, ten miles south of where the Jinja-Mumia's road crosses the Sio river.
3. Uchwenga district.
4. Okala's district, at the mouth of the Nzoia river.
5. Sub-district of Odera Sante; north bank of the Yala near the Lake.

On May 3 we resumed our journey, reaching the Nzoia river about midday. This stream was also in full flood. The porters and baggage were ferried across in a "dug-out" canoe, and the cattle were swum to the other side. After travelling for about eight miles over rich undulating pasture land, supporting numerous herds of cattle and a large human population, we encamped at Lunambo. The chief of the place visited the camp, and on being questioned, he stated that there was no sickness amongst the cattle in the district, and that the banks of the Nzoia river were free from bush or forest for many miles. He was acquainted with the tsetse, and said there were plenty of them about the mouth of the Nzoia.

The following morning—May 4—we resumed our journey, following the old telegraph road for about four miles, until we came to Ukangala, where we left the old road and followed a track across country, which led us into the new road at Adoua, where we encamped for the night. During the day we covered in all about ten miles, travelling over grass land intercepted by swamps and streams choked with papyrus. Huge boulders of grey granite, sometimes piled one upon another, were lying about in all directions, forming a conspicuous feature of the landscape. Dark circles of bush, a clump of trees, or a stout stockade of giant euphorbia, marked the site of a native village, around which grazed herds of cattle and sheep tended by children, whilst all along the road nude men and women were working their patches of "matama" or maize. In the evening, Adoua,

chief of Adoua, who calls himself King of Marachi, came to pay his respects. On being questioned regarding the health of his people and his herds of cattle and sheep, he informed us that many people living near the Lake-shore died of Sleeping Sickness, which he attributed to their eating poisonous fish. Europeans told him that blood-sucking flies frequenting the Lake-shore gave people Sleeping Sickness, but he did not believe it. All the people about Adoua were healthy and had plenty of children and cattle. He knew nothing about flies.

May 5.—Leaving Adoua at 7 a.m., we marched for about two hours, when we were stopped by the Sio river, which we found in flood and utterly impassable for men or animals. We ascertained from a local chief that there was a canoe some six miles up the river towards the north. After walking for many hours through trackless swamps and grass jungle, we reached the ford. The river at this point had overflowed its banks to the extent of half a mile on each side. The cattle were continually getting "bogged," but, eventually, all of them were swum across the ford. Making our way back to the road, we encamped at Mundiza, where there is a substantial rest-house. No tsetse flies were observed in the vicinity of the Sio river. The local chief told us that nearly all the calves born in his district had sickness, and died within three months of birth, but that there was no sickness amongst the grown-up cattle. Three sick calves were brought for our inspection; they appeared to be suffering from East Coast fever, or "Amakebe," as this disease is called in Uganda.

The following day, May 6, we proceeded about five miles along the track until we came to Busire (or Busia). Buagu, chief of the district, an intelligent and enlightened Baganda, informed us that he knew the fly which gave cattle disease, as he had seen it in East Africa when travelling with caravans before the railway was built. He said that this fly formerly existed on the old Kisumu-Jinja road, and that many cattle died after passing along this road, which was now abandoned and overgrown with jungle. He thought we should probably meet with this fly along the so-called "New road," about one day's march from Busire. He told us about a small blood-sucking fly which is very common on the cattle in this district. He did not think it caused disease, because this fly was always with the cattle in many parts where there was no sickness amongst them. On showing him some specimen flies, and requesting him to indicate the fly he referred to, he at once picked out a *Hæmatopota*. No tsetse flies were seen on any part of the road so far traversed. In the country between the Sio river and Busire, *Hæmatopota* are numerous.

May 7.—Marched about sixteen miles to Wakoli's. From Busire onwards the open pasture lands give place to park-like forest country, thinly scattered with acacia bush, with here and there a great forest tree. Luxuriant grass grows everywhere, and there are running streams and swamps every few miles. All along the road swarms of *Hæmatopota* of at least six species—*pulchithorax*, *tenuis*, *similis*, *brunnescens*, *unicolor*, and *nigrescens*—accompanied the cattle, biting incessantly, sucking the blood of men and animals with equal avidity. Whilst we were halting on the banks of a stream between Busire and Bulugwi, one of the fly-boys caught a *Glossina palpalis* feeding from his leg. This stream crosses the road about four miles east of Bulugwi. A belt of forest and thick bush overhangs its banks as far as one could see. According to native information, this brook is an affluent of the Sio river, and is distant from the Lake-shore about half-a-day's march. At Bulugwi there is a substantial rest-house, and beyond this place the road crosses an extensive swamp called Lumbogo. Beyond the swamp the road is again represented by an ill-defined overgrown path, meandering through uninhabited bush and forest.

On May 8 we halted for the day at Wakoli's. In the morning we went out to meet the cattle which had been kraaled the previous night at Bulugwi. They were accompanied by a host of biting flies. No tsetse flies were seen. At Wakoli's, the number of mosquitoes and blood-sucking flies is extraordinary. *Hæmatopota* buzz around the men and cattle all day like bees gathering for a swarm. Seventy were counted settled upon one beast. Many that were captured were distended, and bulged out with blood like a well-filled tsetse fly. Large numbers invaded the tents, biting the people inside, and greedily distending themselves with blood. These

flies are sluggish, doggedly intent on getting blood, regardless of personal safety and, therefore, readily caught. The bite is a painful burning prick, causing no subsequent swelling, pain or inflammation. A smudge of blood frequently marks the place where the insects have fed. They are delicate flies, dying within a few hours of captivity. They are most active from 7 a.m. to midday. When rain falls they disappear. Besides *Hæmatopota* three species of *Tabanidæ*—*thoracicus*, *albipalpus*, and *taniola*—*Stomoxys*, and a very minute vicious little blood-sucking fly, with speckled wings—*Culicoides milnei*—are found in vast numbers at Wakoli's.

May 9.—Shortly after leaving Wakoli's we were confronted by a great unbridged swamp, a mile broad and several miles long. In the absence of any sort of bridge, culvert or bank across or around this morass, we were compelled to cut our way through the papyrus, wading waist-deep in the ooze. Great difficulty was experienced in getting the cattle across; they repeatedly got fixed in the mud, their legs had to be lifted and dug out, and their bodies levered up with poles, until they regained their feet, only to fall and sink again a few yards further on. Whilst in the swamp amidst the cloud of *Hæmatopota* preying upon the cattle, two very large *Tabanidæ* were observed. They were conspicuous by their jet-black abdomen, contrasting against a light-coloured thorax of French grey—*Tabanus biguttatus*. These flies attacked the cattle with great ferocity, but being very active and wary, they eluded capture. Having crossed the swamp and attained higher land, the characters of the landscape changed as we proceeded. Hilly country thickly scattered with great forest trees, and overgrown with elephant grass, succeeded the bush-covered plains and short herbage of the Busire to Wakoli's district. A considerable population, and numerous banana plantations, contrasted with the desolate wilderness left behind. As we progressed through this country the numbers of blood-sucking flies attending the cattle diminished, until only a few small dark-coloured species of *Hæmatopota*—*nigrescens*—were seen on the animals. In the evening we camped at Busesa or Bugwere, a large scattered village surrounded by extensive banana plantations. The local chief reported that there was no cattle disease in his district.

May 10.—After leaving Busesa-Bugwere we followed the footpath known as the "New road" for about seven miles until we came to Iganga, a large native village comprising a market-place and a community of Indian traders. At Iganga we followed the broad highway leading to Jinja, where we arrived on May 11. A small, dark species of *Hæmatopota*—*nigrescens*—were numerous on the cattle all along the road, and a few tabanids were noticed. The "M'bwa" fly (*Simulium damnosum*), is a great pest in this locality. We passed many transport trains of bullock carts and herds of donkeys along the road. Most of these animals were in bad condition, and covered with ticks, which they littered profusely along the road. The number of these distended parasites which were trodden upon and squashed as one walked on the pathway was remarkable. They are, probably, a great source of danger in the spread of tick-borne disease amongst cattle.

From Jinja we proceeded to Mpumu along the well-known Jinja-Kampala highway. No tsetse flies were seen on this road.

Marches and approximate distances travelled:—

	Miles.
April 24-25, Kisumu to Maseno	17
April 30-May 1, Maseno to Mumia's	24
May 2, halt at Mumia's	
May 3-4, Adoua to Mundiza	22
May 6, Mundiza to Busire	5
May 7, morning, Busire to Bulugwi	8
May 7, evening, Bulugwi to Wakoli's	8
May 8, halt at Wakoli's	
May 9, Wakoli's to Busesa	8
May 10, Busesa to Nakalama	11
May 11, Nakalama to Jinja	18
May 12, 14, and 15, Jinja to Mpumu	50
Total	171

Past History of the Cattle.—Forty-eight of the cattle examined were part of a herd of 100 head which were captured from lawless tribes in the Kisi country, on the south shore of Kavirondo Bay, during January and February, 1908. The Kisi country is mostly open pasture land; it extends to the Lake-shore, where it is fringed with a belt of forest and brush infested with *Glossina palpalis*. The cattle came by road through the Kisi country to Kisumu, where they were handed over to the British East Africa Corporation, Ltd. Of the 100 cattle, 50 were grazed at Mumia's Road estate, and 50 at Kibos, on the Kisumu Plain, eight miles from Kisumu. In January, 1909, they were all brought into Kisumu and sold to P. H. Clarke, Esq. They were immediately driven to Mr. Clarke's estate on the Haywood Road, on top of the Escarpment, six miles from Kisumu. They remained at Haywood Road estate from January, 1909, until April, 1909. Fifty of these cattle were purchased by Mr. Drury for the Uganda Company, Ltd., about the middle of April, 1909. During the last year, 1908-09, one beast out of the herd of 100 has died. The cause of death is unknown. Five animals of the herd of 50 purchased by the Uganda Company, Ltd., have been working on transport at Maragoli, on the Escarpment. Two of the herd of fifty came from Mr. Bernson's farm at Bunyore, near Maseno.

Examination of the cattle brought from Kisumu overland to Mpumu.—The blood of the fifty head of cattle was examined in fresh and stained preparations at Mpumu on May 16, 20, and June 1. Seven showed infection with *Trypanosoma ingens*, and one was infected with *Trypanosoma vivax*. Inoculation experiments were not performed. The cattle showed no signs of ill-health during the time that they were under observation at Mpumu. As the animals were exposed to the bites of *Glossina palpalis* between Busire and Bulugwi, and probably at Jinja also, it is not unlikely that the ox infected with *Trypanosoma vivax* contracted the disease on the journey, since *Glossina palpalis* is known to be the carrier of *Trypanosoma vivax*.

CONCLUSIONS.

1. The tsetse fly, *Glossina palpalis*, is found on at least one stream crossing the road between Kisumu and Jinja.
2. The tsetse flies, *Glossina morsitans* and *Glossina pallidipes*, were not found along the "New road."
3. Vast numbers of blood-sucking flies of the genus *Hæmatopota*, *Tabanus*, and *Culicoides* were found along a certain segment of the road six miles to the east and west of Wakoli's.
4. There is no evidence that the cattle contracted any disease from the bites of *Hæmatopota*, *Tabanus*, or *Culicoides*.

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OF THE

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1.—FURTHER RESULTS OF THE EXPERIMENTAL
TREATMENT OF TRYPANOSOMIASIS; BEING A
PROGRESS REPORT TO A COMMITTEE OF THE
ROYAL SOCIETY.*

By H. G. PLIMMER, F.R.S., W. B. FRY, Captain R.A.M.C., and
H. S. RANKEN, Lieutenant R.A.M.C.

The following results are a continuation of the work of which summaries have already appeared in the 'Proceedings of the Royal Society.'† These experiments have been carried out with the same strain of Surra as was used before, at the Brown Institution, and at the Elstree Farm of the Lister Institute.

Further Experiments with Antimony upon Dogs by New Methods.

In the last Report‡ we summarised the results obtained by treating dogs suffering from Surra with antimony. We found that in some dogs the subcutaneous or intramuscular administration of antimony or of its salts caused inflammatory swellings and often abscesses, with much constitutional disturbance, and therefore, although certain good results had been obtained, the method seemed impracticable for the treatment of animals upon a large scale. We then tried the effect of *intravenous* injections of the salts of antimony and even of the metal itself, and this would seem at present to be the most promising method of giving antimony, for, if the injection of the metal into the veins be carried out successfully no irritation is caused. If the salts be injected in solution the elimination is so rapid that no good result can be obtained in the acute form of trypanosomiasis with which we have been working, so the actual injection of the metal itself has been successfully carried out in now a large number of instances. In the last two Reports mentioned above experiments have been recorded with antimony (metal) in a state of extremely fine division, and it was shown that it was relatively much more powerful than the salts in its action upon trypanosomes, and that its effects lasted much longer than those of the salts. The particles of metal, which are very minute—they vary roughly from 0.5μ to 2.5μ —are taken up by the leucocytes, and some compound which is

* Reprinted from the Proceedings of the Royal Society, B. vol. 83 (1911).

† 1907, vol. 79, pp. 500—516; 1908, B. vol. 80, pp. 1—12, and 477—487; 1909, B, vol. 81, pp. 354—371.

‡ 'Roy. Soc. Proc.' B, vol. 81, p. 367.

soluble in the *liquor sanguinis* is apparently formed by them. We are not certain what happens: but in rabbits stained films of blood, taken at various intervals after the intravenous administration of the metal, seem to show that the leucocytes go on taking up the particles until they can contain no more, and then disintegrate at varying periods afterwards, thus providing a constant, though small, supply of antimony. The disintegration would seem to be due to the destruction of the leucocytes by the antimony they have taken up, but on the other hand, many of the leucocytes which have not taken up too much appear to be quite healthy. We have found that both cerebro-spinal fluid and blood serum are, as far as a solution of any trypanocidal substances is concerned, by themselves quite without action on metallic antimony, even when kept in contact with it for periods up to three days. Experiments were undertaken with these fluids after their contact with the metal both on infected animals and upon trypanosomes *in vitro* with entirely negative results.

The time at which no more particles can be found in, or in which they disappear from, the leucocytes, varies in the different animals; in rabbits they can be found for three or four days; in horses we have found them after three days; and in dogs not after two.

Sufficient soluble antimony is formed in all these animals after an appropriate dose of the metal to drive the trypanosomes out of the peripheral blood in about $2\frac{1}{2}$ hours; this is, more than double the time taken by the soluble salts to attain the same result.

We have not had in rabbits, dogs, or horses any plugging of capillaries with the metal, but the animals appear to be more sensitive to over-dosage with the metal than with the salts. Testing the dosage on healthy uninoculated animals is also fallacious, as a fatal dose to a healthy animal is apparently borne well by a similar animal when its blood is full of trypanosomes; we believe, also, that in the same animal a large dose is better borne when the blood contains trypanosomes than when it is free.

When antimony in this form is prepared in larger quantities, it becomes more difficult to remove impurities from it. We mention this as we have had deaths from doses which had previously been well borne, which we attribute to these impurities, but these difficulties are now overcome.

Of the 26 dogs treated with antimony, the details of which are given in the last Report, one (No. 1) lived for over 15 months in good condition, and was then killed. Inoculations from all the organs into rats proved negative, and the animal was regarded as cured; twelve others (Nos. 2, 5, 7, 9, 10, 11, 13, 14, 16, 17, 18, 19), which lived for from 48 to 94 days, if inoculation into rats from the organs after death can be accepted when negative as evidence of cure, may also be regarded as cured.

Average Duration of Untreated Disease, 14 Days.

No.	Weight in kilos.	No. of doses.	Substance used and quantity of dose.	Recur- rences.	Remarks.	Gain or loss of weight.	Subinoculations from organs.
27	9½	18	6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly.	1	<i>Living 1 year 121 days after inoculation</i> ...	kilos. + ½	
28	10¼	9	8 m. 5 per cent. lith. ant. tart. diluted, intramuscularly.	1	Died on 75th day from pneumonia	+3	Negative.
29	9¾	10	7 m. 5 per cent. lith. ant. tart. diluted, intramuscularly.	1	Died on 84th day from pneumonia	+1	Negative.
30	9	12 } 3 }	6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly.	3 }	Died on 168th day with nervous symptoms, "Fits"	-1¼	Positive C.S.F.
31	8½	9 } 3 }	50 mg. ant. intravenously. 6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly.	2 }	Died on 87th day : kidneys inflamed	-1	Negative.
32	11	4 } 2 }	20 m. 5 per cent. lith. ant. tart., diluted, intravenously.	1 }	Died on 154th day, 3 months after last dose. Spleen large.	+1¼	Negative.
33	9	2 } 4 }	80 mg. ant., intravenously 20 m. 5 per cent. lith. ant. tart. intravenously.	1 }	Died on 116th day. Trypanosomes in blood	- ½	Positive.
34	8¼	2 } 4 }	60 mg. ant., intravenously 20 m. 5 per cent. lith. ant. tart., intravenously.	3 }	Died on 81st day from pneumonia	-1¼	Negative.
35	9½	2	80 " "	-	<i>Living 347 days after inoculation</i> ...	+1	Positive.
36	13	4	90 " "	1	Died on 88th day. Trypanosomes in blood	- ½	Negative.
37	9	4	80 " "	2	Died on 67th day with nervous symptoms	-1¼	Negative.
38	7	3	60 " "	2	Died on 40th day. Probably poisoned with ant.	-1½	Positive.
39	9½	5	60 " "	2	Died on 76th day. Probably poisoned with ant.	+1	

The preceding Table shows in outline the results with 13 other dogs since treated with antimony in the method indicated.

The last two dogs probably died from the effects of antimony, as other dogs at the same time died after the first dose of that particular sample.

Dogs are particularly susceptible to both Surra and antimony, but if we accept negative sub-inoculations made from the organs after death as fair evidence of cure, this table and the previous one will work out as follows:—

In the first Dog Table (in the last Report), on this assumption, out of 26 dogs, 13 may be regarded as cured, and in the one above, 2 are alive and 7 gave negative results, that is 22 out of 39 altogether. The indications are also that treatment with the metal intravenously has yielded better results than that with the salts, either intravenously or intramuscularly.

The technique in dogs is difficult, as only the saphenous veins in the hind legs are available without an operation, and the suspension of the metal in salt solution must be as dilute as possible.

Intraperitoneal Injection of Antimony (Metal).

A series of experiments on the effects of intraperitoneal injections of the metal has been made on rats and rabbits. Both bear the metal quite well in the peritoneum, and neither pain nor inflammation were caused. The metal rapidly disappears from the peritoneum, and after three or four days none can be seen. In rats, one dose of 15 milligrammes will keep the trypanosomes out of the blood for an average of 25 days. No loss of weight or appetite occurred. In dogs this method is impracticable, as the injection causes acute peritonitis.

Rats Treated with Arsenophenylglycin and Antimony.

In the last Report is an account of some experiments with arsenophenylglycin. A rat treated with one dose of arsenophenylglycin had a dose of lithium antimony tartrate given to it the day before recurrence took place in other rats treated with arsenophenylglycin. The rat lived 222 days without recurrence: and, regarded as cured, it was reinoculated and treated intraperitoneally with one dose of antimony (metal): it died 32 days after, from a recurrence. Another rat treated with one dose of arsenophenylglycin and five doses of lithium antimony tartrate died of the disease: in this case the latter drug was not given until after recurrence took place.

These experiments show again that no protection is afforded by a previous attack, and also that the time at which treatment is begun is of importance.

New Arsenic and Antimony Compounds.

Five Surra rats were treated with a new arsenic-camphor compound kindly sent to us for trial by Dr. Morgan, of the Imperial College of Science. They died at about the same time as untreated rats, and the substance was found to be too irritating for use.

Eleven Surra rats were treated with a new organic antimony compound, also sent to us by Dr. Morgan. This caused considerable swelling, and did not effect the disappearance of the trypanosomes. The arsenic compound had no effect, the antimony a small effect. *In vitro*, the latter killed the organisms very slowly.

Treatment of Goats with Antimony.

According to Musgrave and Clegg* inoculated Surra in goats is always fatal, and they give 18 days as the time the untreated disease runs.

We have treated two goats with antimony. One has had three doses of about 80 milligrammes each of the metal suspended in egg-yolk and oil subcutaneously, and is living and well 15 to 16 months after inoculation. The other had 15 doses of 20 m. of 5 per cent. lithium antimonyl tartrate, and later, when it became ill with nervous symptoms, four doses of 150 milligrammes of the metal intravenously. It became paralysed and died 236 days after inoculation.

Inoculations into rats from organs and cerebro-spinal fluid were negative.

Treatment of Surra Rabbits with Antimony.

The results of treatment of rabbits with antimony have been striking. They were left until they were very ill with the disease, with sores on the face, swollen genitals, œdema, eyes and nose inflamed, and unable to stand; after treatment all these symptoms disappeared, and their deaths were, we believe, due to poisoning with an impure antimony.

The table on p. 6 sets forth the principal points.

Inoculations made from Nos. 2, 3, and 4 into rats after death were negative.

It will be noticed that after three doses of the salt there was a recurrence, and also after one dose of the metal, but not in those rabbits which had more than one dose of the metal.

* 'Trypanosoma and Trypanosomiasis,' 1903.

No.	No. of doses.	Material used, and quantity.	Recur- rences.	Remarks.
1	3	5 m. 5 per cent. lith. ant. tart., intravenously.	1	Died on 116th day. Recurrence occurred on the 115th day, when the impure metal was given.
2	1	30 mg. ant., intravenously	—	Died on 80th day.
2	3	30 mg. ant., intravenously	—	Died on 183rd day, probably killed with impure antimony. Had increased 300 gr. in weight.
2	1	30 mg. ant., intraperitoneally.	—	Died on 147th day, probably as No. 3. Had increased 180 gr. in weight.
3	2	60 mg. ant., intravenously	—	Died on 79th day. Lost 500 gr. in weight.
4	2	30 mg. ant., intravenously	—	
5	1	30 mg. ant., intravenously	1	

Experiments with Surra Rats after One Dose of Antimony.

We have had the following curious experience with infected rats which had been treated with *one* dose of lithium antimonyl tartrate. One dose of this drug causes the disappearance of all trypanosomes from the blood, and keeps the blood free from them for a variable period, the average time of recurrence being 18 days. We wished to find out how soon the blood of a rat thus treated became infective to other rats on sub-inoculation, and whether it would prove to be so before trypanosomes could be found in it by ordinary microscopic examination. Sub-inoculations were therefore made from the treated rat's blood at various intervals from the 2nd to the 16th day after the one dose of antimony. It was then found that the blood of the original rat was infective long before any trypanosomes could be found in the peripheral circulation; but also that this infectivity was not constant, an infection being produced on one day, and none occurring on one or two subsequent days. Moreover, as the table shows, the course of the disease (incubation period especially) was very prolonged. Ordinary untreated infective blood produces in rats a recognisable infection in from two to four days, and death is from six to seven days. In these cases either the trypanosomes could not be found in the peripheral blood at all, the only lesion being the very large spleen, or were not found until the 28th day after inoculation. These prolonged incubation periods appeared to have no relation to the time of the recurrence in the original rat, and the infection was brought about by blood which not only showed no trypanosomes, but appeared to be perfectly normal in structure. We have no explanation of these results, but as all the rats which died had very large spleens we can only suppose that the trypanosomes were present in either some form or place which we did not recognise or find. If this be so the experiments are of interest as showing the influence on the trypanosomes, and the constant effects, of one dose of antimony.

Table giving Details of the above-mentioned Experiments.

Rats inoculated from treated Rat 1 on—	Death occurred on—	Spleen.	Trypanosomes present (+) or absent (0) from blood.	Remarks.
5th day after dose	—	—	—	Living 103 days.
7th " "	31st day	Very large	0	
10th " "	16th day	Large	0	
12th " "	—	—	—	Living 95 days.
14th " "	13th day	Very large	0	
Ditto from Rat 2 on—				
2nd day after dose	—	—	—	Living 52 days.
4th " "	—	—	—	Living 50 days.
7th " "	—	—	—	Living 47 days.
10th " "	28th day	Very large	++	
16th " "	41st day	Enormous	0 in blood, spleen, liver, adrenals.	

It will be noticed that death was delayed up to as long as 41 days. Two other rats were given a relatively smaller dose of antimony, and rats inoculated from these on the 4th, 7th, 9th, and 11th days after the dose died with trypanosomes in their blood on the 24th, 19th, 19th, and 29th days respectively, the incubation period only being much prolonged.

Experiments upon Surra Horses with Antimony.

Inoculated Surra in horses runs a quicker course than that acquired naturally, and it is difficult to give a certain time for the disease, as a good deal depends on the condition of the horse, and experimental horses are usually old and worn-out and would succumb more quickly than young ones. Cases of untreated experimental Surra in horses have died as early as the 14th day, but the time probably depends very much upon the age and condition of the animal. The horses we have used were inoculated from rats, and the incubation period was practically six days. Musgrave and Clegg* give 6 to 13 days as the incubation period, so that our strain of Surra is a fairly virulent one for horses.

Both intramuscular and intravenous injection of antimony (metal) have been tried. Doses of 2 grammes have been given, intravenously, and if sufficiently diluted with warm salt solution no unpleasant symptoms follow. Large doses are best borne when injected into a peripheral vein in one of the legs, and one injection of 1 gramme will keep the blood free from trypanosomes for from 2 to 3 weeks. If the injection be made into the jugular vein considerable depression, and even faintness, may be induced. The

* *Op. cit.*

use of a peripheral vein involves throwing the horse, which is risky if repeated doses have to be given. We have not yet ascertained the maximum dose. All the horses died with nervous symptoms, and although life has been prolonged (in one case to 121 days) none of them has shown satisfactory results. We have persisted in our experiments with metallic antimony because we hoped that, as it is taken up by the leucocytes, it might possibly be carried by them to, and be deposited in, parts not accessible otherwise; also, because we know that in syphilis the protozoon causing the disease is killed by the injection of metallic mercury. We know also that syphilis requires a continued treatment, and it may be that in trypanosomiasis a continued treatment also is necessary. The effects produced by metallic antimony are so striking that we are emboldened to think that if the methods could be improved a much better effect would be obtained.

The following Table summarises the experiments upon horses :—

No.	No. of doses.	Material and quantity given.	Recur- rences.	Remarks.
1	7	Intramuscular— 20 per cent. suspension of sod. ant. tart. in Lambkin's medium. Doses from 1 to 20 c.c.; in all 36 c.c.	—	No trypanosomes seen for 17 days before death. Died on 44th day with nervous symptoms and thoracic cedema. Spleen normal.
	1	5 c.c. 5 per cent. ant. in Lambkin's medium.		
2	8	Intravenous— Ant. in salt solution. Doses from 0·5 gr. to 1·25 gr. altogether 5·5 gr.	2	No trypanosomes seen for 19 days before death. Died on 52nd day with nervous symptoms.
3	8	Ant. in salt solution. Doses from 0·1 gr. to 2 gr., altogether 9·75 gr.	3	No trypanosomes seen for 20 days before death. Died on 121st day with nervous symptoms.
4	6	Ant. in salt solution. Doses from 0·1 gr. to 2 gr., altogether 4·5 gr.	—	No trypanosomes seen after first dose. Died on 56th day with nervous symptoms. Had persistent thoracic cedema.

With the exception of No. 1, these horses were old and worn-out

It will be noticed that none of these horses had trypanosomes in the blood for some time before death; No. 3 appeared to be quite well up to the 90th day, and No. 4 up to the 40th day, and both could apparently have worked up to these times. In all cases death was preceded by a very sudden aggravation of symptoms referable to the nervous system.

We considered that treatment by injection into the cerebro-spinal space was impracticable in horses owing to the distance of the spinal canal from the surface, and to the fact that the spinal cord extends much further down the canal than in man.

Experiments with Silver Salts.

Three Surra rats were treated with protargol in doses varying from 0.5 c.c. of a 1-per-cent. solution to 1 c.c. of a 2-per-cent. solution.

Three Surra rats were treated with argyrol in similar doses.

Three Surra rats were treated with silver nitrate in doses varying from 1 c.c. of a 0.02-per-cent. solution to 1 c.c. of a 1-per-cent. solution.

All these rats died at the usual time: death was not delayed, nor were the trypanosomes reduced in number in the blood. *Post-mortem* examination showed that they had died with the usual signs of acute trypanosomiasis, with very large spleens, etc. At the sites of injection of protargol there was œdema and brown staining of the tissues in a circumscribed area: there was more marked œdema and swelling after injection of argyrol, and œdema and localised necrosis of the tissues after injection of silver nitrate. It would appear that the silver salts are not absorbed from the site of injection. Presumably they have a primary cauterising action on the tissues, and are quickly transformed into silver chloride, which is inert.

In vitro experiments were carried out with these three compounds, all of which are soluble in distilled water. Equal sized drops of blood containing trypanosomes and of the solutions were mixed, and the results observed under the microscope.

Protargol solutions from 1/200 to 1/10000 produced very little effect; in 1/200 dead trypanosomes were seen in 30 minutes, but many were alive in 24 hours. Silver nitrate in solutions from 1/200 to 1/10000 has much the same effect as protargol.

Some trypanosomes are killed in 30 minutes by the stronger solutions, but the remainder are as active as the controls up to 24 hours and longer.

Argyrol (which contains 30 per cent. silver) in solutions of 1/1000 to 1/10000. The stronger solutions are still less active than those of protargol and silver nitrate; and the more dilute have no effect up to three hours. Thus it appears that these bodies have no effect except the early effects of the stronger solutions; they probably combine with the salts in the blood-plasma and corpuscles, and become inert.

Citrate of silver in its strongest solution of 1/4000 is also inert.

2.—NOTE UPON THE EXAMINATION, WITH NEGATIVE RESULTS, OF THE CENTRAL NERVOUS SYSTEM IN A CASE OF CURED HUMAN TRYPANOSOMIASIS.*

By FREDERICK W. MOTT, M.D., F.R.S.

This is the first case in which "a chance has occurred of examining a cured case of human trypanosomiasis *post mortem*," according to the statement of C. A. Wiggins, the Acting

* Reprinted from the Proceedings of the Royal Society, B. vol. 83.

Principal Medical Officer of the Uganda Protectorate, who has kindly forwarded me the tissues for microscopic examination.

Summary of the History of the Case.—Narain Singh, a Sikh, belonging to the 4th K.A.R. (aged 30 at death), was found to be suffering from trypanosomiasis in June, 1905, and received treatment with inorganic arsenic. The previous history as far as ascertainable, compiled from extracts of reports and history sheets, is published in the full account, but, in brief, it may be assumed that the drug was given intermittently for 18 months or more, and pushed till toxic symptoms of neuritis, mental dullness, etc., rendered further energetic treatment impossible; trypanosomes were then no longer obtained by puncture of the glands. Unfortunately there is no note of lumbar puncture having been performed until a few months before death, therefore we do not know whether invasion of the sub-arachnoid space ever occurred either before or after the treatment. But it is probable that trypanosomes were never present in the cerebro-spinal fluid, for if they were, the symptoms of Sleeping Sickness would, in all probability, have come on, in spite of treatment by atoxyl. Sir David Bruce, in December, 1908 (that is three and a half years after the trypanosomiasis had been discovered), saw this man, and stated that he appeared to be in excellent health. A year later he was seen by Captains Hamerton and Bateman, who reported no symptoms of Sleeping Sickness. They made a very careful investigation of the blood, both by microscopic examination and by experimental injection into monkeys; the results were negative. In June, 1910, lumbar puncture was performed, and 17 c.c. of fluid withdrawn; the centrifuged fluid showed no lymphocytosis or trypanosomes; and injection of the fluid into a monkey was followed by negative results. The patient was attacked with pneumonia in August, 1910, and died three days after admission to the hospital. At the *post-mortem* examination grey hepatisation of the whole right lung was discovered. Beyond fibrosis and induration of the cervical and inguinal glands there was nothing noteworthy in the appearance of the organs and tissues. The brain was quite normal in appearance, and there was no excess of fluid.

It may be mentioned that this man contracted syphilis on July 27, 1906, and he was treated for some time with mercury, and a note on February 15, 1908, is as follows:—Condition the same as August 6, 1907, but no trypanosomes found in blood or glands, due to fact of taking mercury for syphilis.

Histological Examination.—Sections were prepared of portions of the cerebrum, cerebellum and medulla oblongata, by all the methods which I have previously adopted for the examination of the tissues of Sleeping Sickness cases. I found no trace of the characteristic meningeal and perivascular infiltration, nor of gliosis. Sections of the spleen, liver, and kidney were also examined with negative results.

I have shown that there is a parallelism between the intensity of the signs and symptoms of Sleeping Sickness and the diffuseness and intensity of the lymphatic perivascular infiltration with lymphocytes and plasma cells which is the main cause of the clinical phenomena.

It may be asserted that this case proves that human trypanosomiasis is curable, but it does not prove that Sleeping Sickness is curable, for I contend that the diagnosis of "Sleeping Sickness" can only be made when there is proof that the trypanosomes have invaded the sub-arachnoid space. The case emphasises the importance of early diagnosis of the infection and the value of early energetic treatment by organic arsenic preparations. How far the administration of mercury assisted to complete the cure is uncertain.

UGANDA PROTECTORATE.

Death Report.

Entebbe Station, August 17, 1910.

Name.	Nationality.	Age.	Sex.	Date of admission.	Date of death.	Disease for which admitted.	Cause of death.	Place of death.
Narain Singh, No. 4662 I.C. 4th K.A.R.	Sikh	30	Male	3.8.10	6.8.10	Bronchitis.	Pneumonia.	Indian Contingent, 4th K.A.R. Hospital.

Previous history as far as ascertainable, compiled from extracts of reports and history sheets:—

22.6.05. Trypanosomiasis. Received treatment inorganic arsenic.

4.12.06. Gland puncture. Trypanosomes +.

Four injections 2 c.c. atoxyl 20 per cent. December 6, 7, 16, and 17.

18.12.06. Glands trypanosomes +.

28.12.06. Glands trypanosomes —. Atoxyl 20 per cent., 2 c.c.

29.12.06. Repeated atoxyl 20 per cent., 2 c.c.

30.1.07. Glands trypanosomes —.

8.2.07. Glands trypanosomes —.

Condition.—Arsenical neuritis. Drowsy looking. Rheumatic pains.

6.8.07. Condition far from satisfactory. Irregular fever, rapid pulse. Dull and stupid. Cervical glands enlarged. Trypanosomes not found in glands since March, 1907. Energetic treatment with arsenic impossible owing to symptoms of poisoning rapidly appearing.

15.2.08. Condition the same 6.8.07, but no trypanosomes found in blood or glands, due to the fact of taking mercury for syphilis (contracted 27.7.06).

16.12.08. *General Conditions.*—Strong, well nourished. No tremors. Reflexes normal.

Gland puncture and blood examination trypanosomes —.

Differential count. Lymphocytes 70 per cent.

Some tendency to drowsiness when unoccupied.

Last treatment, two injections kharsin, May, 1908.

18.12.08. Proceeded to Mpumu to do duty with the Royal Society's Sleeping Sickness Commission. Report by Sir David Bruce—"This man appears to be in excellent health." Returned to Entebbe.

16.12.09. Report by Captains Hamerton and Bateman, R.A.M.C., Sleeping Sickness Commission, Mpumu—"No symptoms of Sleeping Sickness."

Blood Examination.—Examined peripheral blood. Negative results 14 occasions.

Examined centrifuged peripheral blood. Results negative.

Examined blood from median basilic vein. Results negative.

Examined blood from median basilic vein centrifuged. Results negative.

16.12.09. 4 c.c. blood from median basilic injected into monkey.

29.12.09. 5 c.c. blood from median basilic injected into monkey.

1st monkey examined 15 times. Results negative.

2nd monkey examined 12 times. Results negative.

13.6.10. Lumbar puncture 17 c.c. Cerebro-spinal fluid abstracted centrifuged. Trypanosomes —. No excess white cells. 12 c.c. injected into monkey. Monkey examined twice weekly over one month. Results negative.

General habits.—Intemperate. Had gonorrhœa and syphilis.

Post-mortem appearances 14 hours after Death.

Body of lean but in no way emaciated man.

Externally.—Cervical glands—small, hard, and shotty. Inguinal glands—small, hard, and shotty.

Chest.—Parietal and visceral pleura adherent right side, due to recent pleurisy.

Right Lung.—Weight 58 ozs. On section the whole lung was in a state of grey hepatisation.

Left Lung.—Weight 14 ozs. Pleura and lung substance normal.

Heart.—Weight 12 ozs. Right heart distended with blood clot. All chambers and valves normal. The glands at the root of lungs and around bronchi and trachea were enlarged and soft, and some were purulent.

Microscopic.—Examination of this pus showed organisms resembling pneumococci.

Liver.—Weight 89 ozs. Enlarged and congested. On section, soft and friable. Probably some fatty change. No obvious fibrotic changes.

Portion preserved for microscopic examination in England.

Spleen.—Weight 14 ozs. Feels hard to the knife on section. Pale appearance and excess of fibrous tissue on cutting open.

Portion preserved for microscopic examination in England.

Kidneys.—Right, weight 5 ozs. Left, 6 ozs. Both were swollen, the kidney substance bulging from the capsule when cut into.

Both capsules were a little adherent.

Cortices undiminished. The whole kidney substance appeared a little paler than normal.

Portions preserved for microscopic examination in England.

Pancreas and Suprarenals.—Normal.

Stomach.—Slightly dilated. No gastritis. Some *post-mortem* staining.

Intestines.—Normal. Mesenteric and retroperitoneal glands not enlarged.

Brain.—Calvaria normal. Weight of brain, 52 ozs. No thickening of membranes. Brain substance normal. Ventricles normal in size and no excess of fluid.

Portions of cerebral cortex and cerebellum preserved for examination in England.

(Signed)

H. B. OWEN,

Medical Officer, Civil Hospital, Entebbe.

3.—THE MORPHOLOGY OF *TRYPANOSOMA EVANSI* (STEEL).*

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service.

[Plate 1.]

Introduction.

In previous papers published in the 'Proceedings,' the morphology of various trypanosomes, such as *Trypanosoma pecorum*, *vivax*, *uniforme*, *nanum*, and *brucei*, has been described somewhat more fully than is usually done. It is proposed to do the same for *Trypanosoma evansi* in this paper.

This trypanosome causes the disease in elephants, camels, horses, cattle, and dogs, known in India as Surra. It was discovered in 1880, by Evans, in the Punjab.

A. *Living, Unstained.*

Trypanosoma evansi is described by Laveran and Mesnil as being more motile than *Trypanosoma brucei*, and as sometimes travelling out of the field of the microscope, which is rarely the case with *Trypanosoma brucei*.

B. *Fixed and Stained.*

The blood films were fixed, stained, and measured as previously described in the 'Proceedings.'†

Length.—The following table gives the length of this species as found in the elephant, camel, horse, dog, guinea-pig, and rat.

It will be seen from the following table that *Trypanosoma evansi* varies in length between 18 and 34 microns, the average of 820 individuals being 24·9. It is true that short, stumpy individuals, without free flagellum, are sometimes found, but very few of these rare specimens were met with in the 820 trypanosomes taken as they came for the purposes of the table.

* Reprinted from the Proceedings of the Royal Society, B. vol. 83,

† 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 16 and 17.

These short and stumpy forms are so few and far between that this species may be described as monomorphic, whereas *Trypanosoma brucei*, which has about 40 per cent. of these short stumpy forms, may be called dimorphic.

Breadth.—This lies usually between 1·5 and 2 microns.

Shape.—*Trypanosoma evansi* resembles very closely the intermediate forms of *Trypanosoma brucei*. The posterior extremity is often triangular in shape, the body gradually narrowing towards the anterior extremity (Plate 1).

Table I.—Measurements of the Length of *Trypanosoma evansi*.

No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
—	Elephant	—	—	27·0	32·0	20·0
—	"	—	—	26·5	31·0	21·0
—	"	—	—	26·7	31·0	21·0
—	"	—	—	26·1	29·0	22·0
—	"	—	—	27·3	32·0	23·0
—	Camel	—	—	22·4	28·0	18·0
—	"	—	—	24·0	27·0	20·0
—	"	—	—	23·4	28·0	19·0
—	"	—	—	22·9	27·0	18·0
—	"	—	—	22·6	27·0	18·0
184	Horse	—	—	26·2	28·0	22·0
184	"	—	—	25·9	29·0	21·0
184	"	—	—	25·0	29·0	18·0
184	"	—	—	25·1	30·0	19·0
—	"	—	—	22·9	27·0	20·0
—	"	Osmic acid	Giemsa	25·3	29·0	18·0
14	Dog	—	—	25·2	28·0	22·0
1	"	—	—	26·7	30·0	21·0
—	"	—	—	24·7	29·0	21·0
2	"	—	—	26·3	30·0	23·0
—	"	—	—	25·7	28·0	24·0
—	Guinea-pig (1)	Osmic acid	Giemsa	24·5	27·0	22·0
—	" (2)	"	"	24·4	29·0	21·0
—	" (3)	"	"	24·4	27·0	22·0
—	" (4)	"	"	24·6	27·0	22·0
166	"	—	—	23·9	28·0	21·0
87	"	—	—	27·4	34·0	21·0
—	"	—	—	27·2	31·0	24·0
191	"	—	—	23·5	27·0	19·0
—	"	—	—	26·8	31·0	25·0
—	Rat (1)	Osmic acid	Giemsa	24·8	28·0	21·0
—	" (1)	"	"	22·7	25·0	20·0
—	" (2)	"	"	24·7	29·0	21·0
—	" (2)	"	"	23·2	25·0	20·0
—	"	—	—	24·1	29·0	21·0
—	"	—	—	24·3	27·0	19·0
—	"	—	—	24·3	28·0	19·0
—	"	—	—	26·5	32·0	22·0
—	"	—	—	26·6	29·0	24·0
131	"	—	—	25·1	27·0	22·0
—	"	—	—	23·4	26·0	20·0
				24·9	34·0	18·0

Table II.—Distribution in respect to Length of 820 Individuals of *Trypanosoma evansi*.

Animal.	Microns.																	Average Length.
	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	
Elephant...	...	...	1	—	1	1	—	3	2	1	2	3	—	3	1	—	—	27.0
"	...	...	—	1	1	1	—	2	3	3	6	5	—	2	1	—	—	26.5
"	...	...	—	—	2	—	3	3	—	3	2	1	—	—	—	—	—	26.7
"	...	...	—	—	—	—	6	3	3	2	3	5	—	2	1	—	—	26.1
Camel	1	2	2	2	—	2	3	1	—	—	1	—	2	—	—	—	—	27.3
"	...	...	—	3	2	3	3	5	3	—	2	—	—	—	—	—	—	22.4
"	...	...	2	2	3	1	3	—	4	—	1	—	—	—	—	—	—	24.0
"	...	...	—	—	—	3	5	—	2	—	—	—	—	—	—	—	—	23.4
Horse	...	...	—	—	1	3	5	—	1	1	3	—	—	—	—	—	—	22.9
"	...	...	—	—	1	2	1	3	2	—	7	4	—	—	—	—	—	22.6
"	...	...	—	1	4	1	3	1	4	2	5	3	—	—	—	—	—	26.2
"	...	...	—	1	1	1	4	1	3	3	2	1	—	—	—	—	—	25.9
"	...	...	—	5	1	2	2	2	4	2	4	3	1	—	—	—	—	25.0
Dog	1	—	3	—	1	2	2	1	3	3	4	—	—	—	—	—	—	25.1
"	...	...	—	—	2	2	—	3	2	—	2	1	—	—	—	—	—	25.3
"	...	...	—	—	—	—	7	—	3	—	9	—	—	—	—	—	—	25.2
"	...	...	—	1	1	1	1	1	7	1	4	1	—	—	—	—	—	26.7
"	...	...	—	1	—	2	2	5	3	2	4	2	—	—	—	—	—	24.7
"	...	...	—	—	—	—	3	—	6	—	2	1	—	—	—	—	—	26.3
Guinea-pig	...	...	—	—	—	—	1	—	8	—	3	—	—	—	—	—	—	25.7
"	...	...	—	—	—	—	4	3	1	1	4	—	—	—	—	—	—	24.5
"	...	...	—	3	2	5	1	6	1	2	—	1	—	—	—	—	—	24.4
"	...	...	—	—	3	3	6	4	3	2	—	—	—	—	—	—	—	24.4
"	...	...	—	—	—	7	2	4	2	3	—	—	—	—	—	—	—	24.6
"	...	...	—	—	—	1	1	1	2	1	1	2	—	—	—	—	—	23.9
"	...	...	—	1	—	—	3	2	3	5	5	4	2	2	2	1	1	27.4
"	...	...	—	1	1	—	—	4	1	1	5	—	—	—	—	—	—	27.2
"	...	...	—	—	—	6	—	3	3	2	3	1	—	—	—	—	—	23.5
Rat	...	...	—	1	—	—	—	6	4	—	4	—	—	—	—	—	—	26.8
"	...	...	—	—	—	3	3	7	—	—	1	—	—	—	—	—	—	24.8
"	...	...	—	3	1	5	6	2	2	—	1	—	—	—	—	—	—	22.7
"	...	...	—	1	3	2	2	4	4	—	—	—	—	—	—	—	—	24.7
"	...	...	—	—	6	4	7	4	—	—	—	—	—	—	—	—	—	23.2
"	...	...	—	—	3	3	4	7	—	—	—	—	—	—	—	—	—	24.1
"	...	...	—	1	3	1	3	4	4	—	—	—	—	—	—	—	—	24.3
"	...	...	—	2	2	1	3	4	3	2	1	—	—	—	—	—	—	24.3
"	...	...	—	—	1	1	2	2	4	5	1	—	—	—	—	—	—	26.5
"	...	...	—	—	—	—	2	3	3	5	6	1	—	—	1	—	—	26.6
"	...	...	—	—	—	—	4	—	8	—	—	—	—	—	—	—	—	25.1
"	...	...	—	—	—	—	2	—	3	—	—	—	—	—	—	—	—	23.4
Totals	...	6	10	21	40	59	75	130	125	108	118	70	35	9	3	—	1	
Percentages	...	0.7	1.3	2.5	4.9	7.1	9.2	15.8	15.3	13.1	14.4	8.5	4.3	1.1	0.4	—	0.2	

Contents of Cell.—According to Sir John McFadyean, a large proportion of *Trypanosoma evansi* show no distinct granules in front of the nucleus, whereas the majority of *Trypanosoma brucei* do. Moreover, when granules are present in the former, they are, as a rule, not so large or so numerous or so deeply stained as they are in the latter. These differences are not distinct except in well stained preparations, and Giemsa or some other Romanowsky method is the best for the purpose.

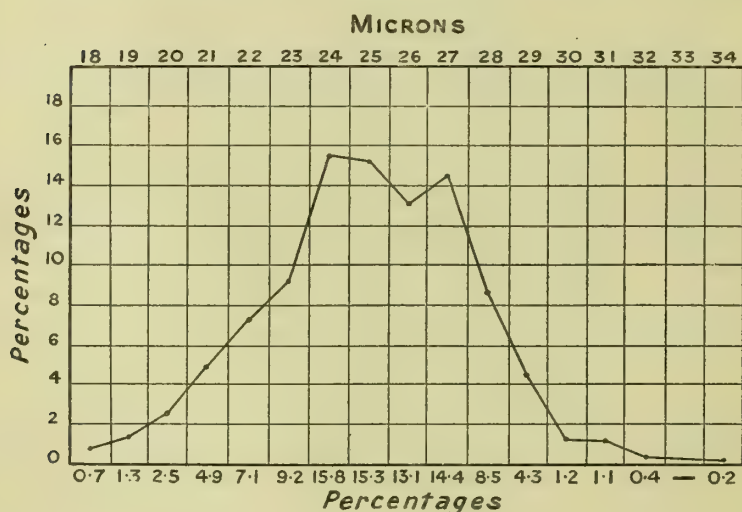
Nucleus.—Is oval or round and situated about the middle of the body.

Micronucleus.—Small and round, situated, on an average, 1·5 microns from the posterior end; maximum, 4, minimum, 0·5.

Undulating Membrane.—Is well developed and thrown into folds.

Flagellum.—Most of these trypanosomes have a free flagellum. It averages 4 microns in length: maximum, 9, minimum, 1. A small percentage are without free flagellum, but this, in my experience, is rare.

Chart 1.—Chart giving Curve representing the Distribution, by percentages, in respect to Length of 820 Individuals of *Trypanosoma evansi*.

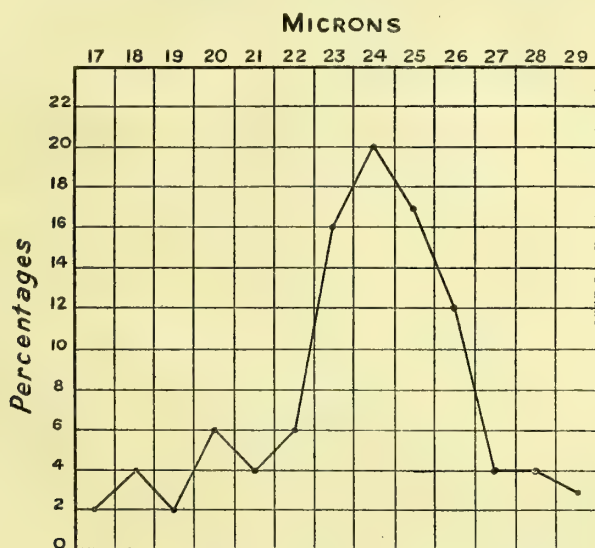


Comparison of the Disease of Camels, Sudan, with that of India.

The disease of camels in the Sudan called Mbori is considered by Laveran and others to be caused by *Trypanosoma evansi*. It will therefore be interesting to compare a curve of the camel disease in India with the camel disease of the Sudan. I am indebted to Dr. Andrew Balfour, Khartoum, for a series of slides

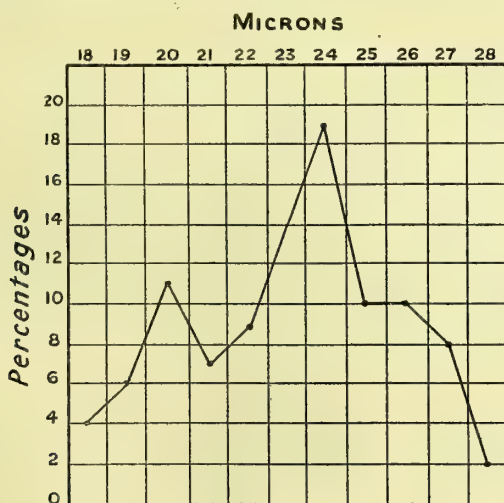
of this trypanosome, from which the following curve has been made:—

Chart 2.—Chart giving Curve representing the Distribution, by percentages, in respect to Length of *Trypanosoma evansi*. Sudan Camel Disease.

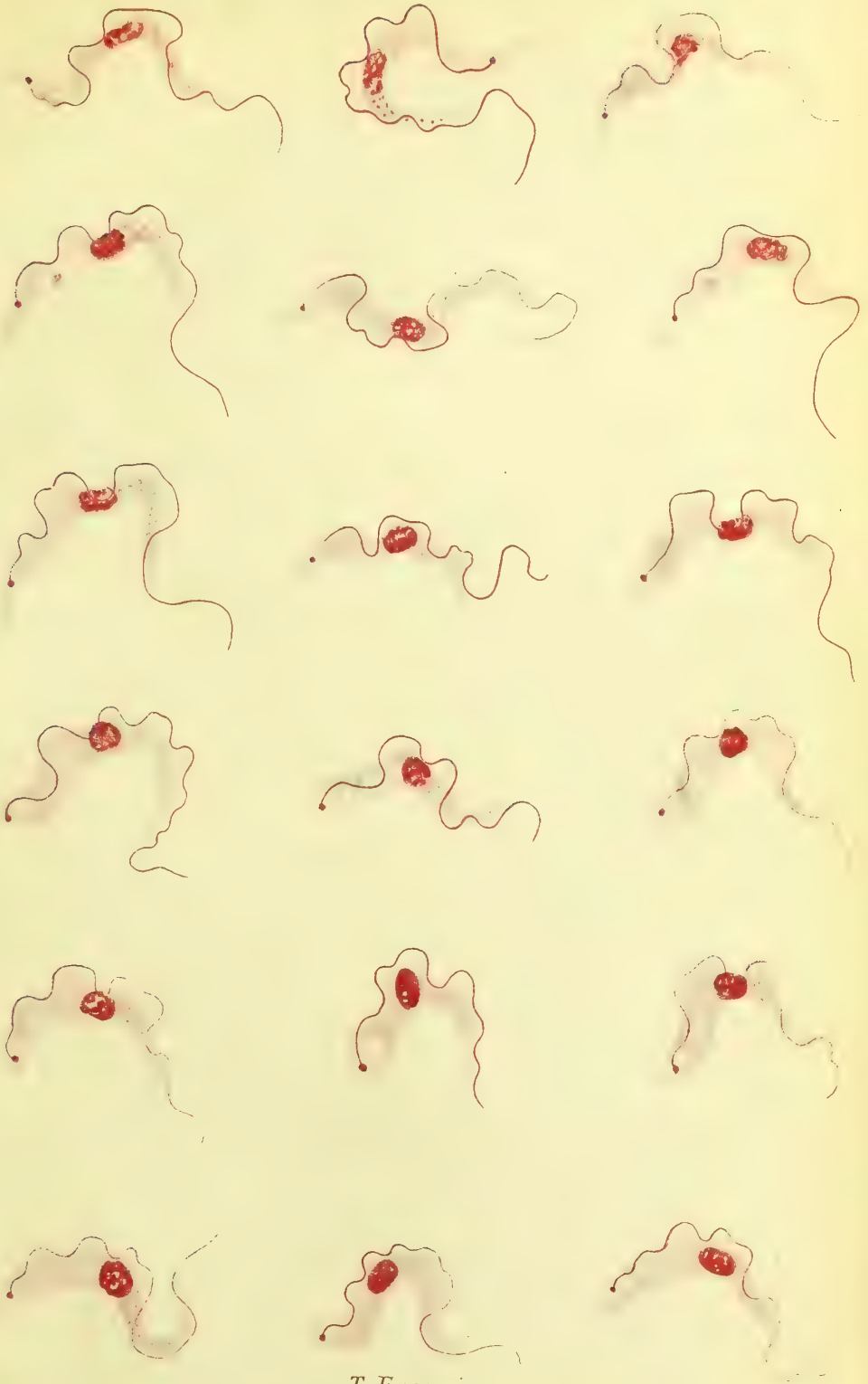


The next chart represents the curve of the Indian camel disease, and I am indebted to Mr. J. D. E. Holmes, M.A., D.Sc., Imperial Bacteriologist, Muktesar, India, for the slides from which the curve has been constructed:—

Chart 3.—Chart giving Curve representing the Distribution, by percentages, in respect to Length of *Trypanosoma evansi*. Indian Camel Disease.



The similarity of these two curves is very striking, and affords some proof that the camel disease of India and that of the Sudan is caused by the same species of trypanosome.



T. Evansi.

But, as we have seen, if a curve is made of the distribution of length among individuals of the species *Trypanosoma evansi* and compared with a similar curve of *Trypanosoma brucei*, then the difference between the two is striking.

DESCRIPTION OF PLATE 1.

Trypanosoma evansi from the blood of various animals, fixed osmic acid, stained Giemsa. $\times 2000$.

Note the similarity in appearance between this trypanosome and *Trypanosoma brucei*.* The nucleus is oval, or round, and is placed near the middle of the body. The micro-nucleus is small and round. One short and stumpy trypanosome, without free flagellum, is shown.

4.—THE MORPHOLOGY OF TRYPANOSOMA GAMBIENSE (DUTTON).†

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.

(Received September 29,—Read November 2, 1911.)

[Plate 2.]

Introduction.

This species, like *Trypanosoma brucei*, is markedly dimorphic. In size and general appearance also these two species so closely resemble one another that one might easily believe them to be varieties of the same species. There are, however, some slight differences in morphology, which will be described below; but whether these differences will bear the test of more extended observations remains to be seen. It may be noted that the trypanosomes described come from Uganda, and are not mixed up with strains from the Congo or Rhodesia.

A. Living, Unstained.

Trypanosoma gambiense also resembles *Trypanosoma brucei* in having little or no translatory power when viewed alive in the field of the microscope.

B. Fixed and Stained.

The blood films were, as a rule, fixed, stained and measured as previously described in the 'Proceedings.'‡

Length.—The following table gives the length of this trypanosome as found in man, chimpanzees, monkeys, oxen, antelope and rats, 1,000 trypanosomes in all. (See Table I.)

From the following table it would appear that *Trypanosoma gambiense* is somewhat smaller than *Trypanosoma brucei*, which was found to average 23·2 microns in 1,000 individuals, with a maximum length of 38 and a minimum of 13. (See Table II.)

Great differences are sometimes found in the average length of the trypanosomes in the same individual. For example, in Experiment 114, man (J.M.), on one day, at the beginning of his illness,

* 'Roy. Soc. Proc.,' 1910, B, vol. 83, Plates 1 and 2.

† Reprinted from the Proceedings of the Royal Society, B, vol. 84, 1911.

‡ 'Roy. Soc. Proc.,' B, 1909, vol. 81, pp. 16 and 17.

the average of 20 trypanosomes was only 17·0 microns; whereas, on another day, at a later date, this rose to 25·8 microns.

Table I.—Measurements of the Length of *Trypanosoma gambiense*, Uganda Strain.

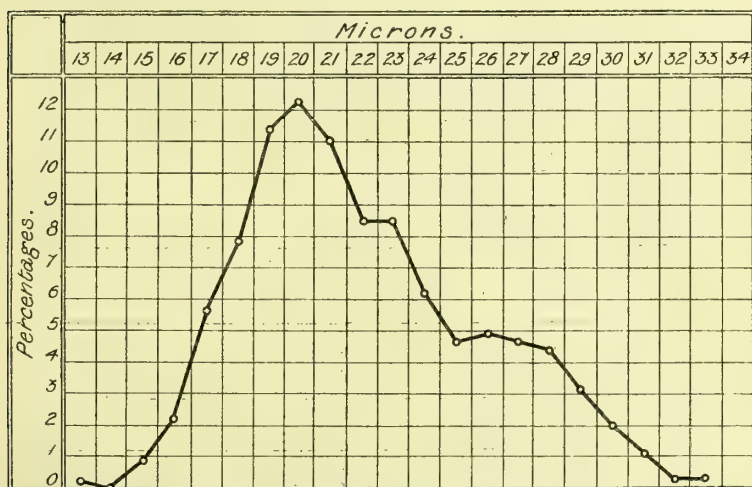
No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
114	Man (J. M.)	Osmic acid	Leishman	17·0	20·0	15·0
114	" "	"	"	25·8	30·0	20·0
103	" (L. S.)	"	"	25·0	32·0	16·0
103	" "	"	"	25·7	32·0	19·0
—	"	"	"	28·3	33·0	21·0
—	Chimpanzee	"	"	22·0	29·0	18·0
—	"	"	"	24·6	33·0	19·0
—	"	"	"	23·7	33·0	18·0
579	Monkey	"	Giemsa	22·5	31·0	17·0
674	"	"	"	21·5	28·0	15·0
976	"	"	"	24·8	29·0	18·0
976	"	"	"	22·8	31·0	17·0
1417	"	"	"	21·0	25·0	18·0
1418	"	"	"	21·4	26·0	16·0
1423	"	"	"	19·6	26·0	17·0
1423	"	"	"	20·3	30·0	16·0
1424	"	"	"	23·7	30·0	18·0
1424	"	"	"	22·7	29·0	17·0
1685	"	"	"	22·2	28·0	19·0
1685	"	"	"	23·5	30·0	17·0
—	"	"	"	20·2	27·0	17·0
1277	"	"	"	24·7	31·0	18·0
2483	"	"	"	26·2	30·0	20·0
982	Ox	"	"	19·3	23·0	17·0
1462	"	"	"	20·1	29·0	17·0
1462	"	"	"	18·9	23·0	16·5
1633	"	"	"	19·0	26·0	16·0
1633	"	"	"	19·0	25·0	15·0
1201	"	"	"	19·7	25·0	17·0
982	"	"	"	20·8	24·0	18·0
2431	Reedbuck	"	"	24·7	30·0	17·0
2359	"	"	"	22·9	28·0	20·0
2429	"	"	"	21·7	30·0	18·0
2445	"	"	"	20·7	27·0	15·0
2357	"	"	"	21·6	25·0	18·0
2429	"	"	"	22·7	27·0	18·0
2371	Bushbuck	"	"	19·5	22·0	16·0
2372	"	"	"	19·9	24·0	16·0
2371	"	"	"	20·8	24·0	18·0
2327	"	"	"	22·1	27·0	19·0
2371	"	"	"	20·8	24·0	19·0
2372	"	"	"	21·5	26·0	19·0
2484	Rat	"	"	19·4	27·0	15·0
2494	"	"	"	19·6	26·0	16·0
XC	"	"	"	22·4	30·0	18·0
2457	"	"	"	21·4	30·0	16·0
24	"	"	"	21·4	29·0	17·0
129	"	"	"	25·4	32·0	18·0
201	"	"	"	23·6	29·0	13·0
—	"	"	"	26·3	31·0	16·0
				22·1	33·0	13·0

The average length of *Trypanosoma gambiense* in man and the other species of animals, taken from Table I, is as follows:—

Table II.

Species of animal.	In microns.		
	Average length.	Maximum length.	Minimum length.
Man	24·3	33·0	15·0
Chimpanzee	23·4	33·0	18·0
Monkey	22·4	31·0	15·0
Ox	19·5	29·0	15·0
Reedbuck	22·4	30·0	15·0
Bushbuck	20·7	27·0	16·0
Rat	22·4	32·0	13·0

CHART I.—Chart giving Curve representing the Distribution, by Percentages, in respect to length of 1,000 Individuals of *Trypanosoma gambiense*.



Breadth.—The long and slender forms average 1·5 microns, the short and stumpy 2·5 microns.

Shape.—This, as stated above, is a markedly dimorphic species.

Contents of Cell.—The protoplasm often shows many chromatin granules in its substance.

Nucleus.—Resembles *Trypanosoma brucei*, in that the nucleus is oval in the long and slender, and round in the short and stumpy forms.

Micronucleus.—Small and round, and situated, on an average, 1·1 microns from the posterior extremity in the short and stumpy, 1·3 in the intermediate, and 1·8 in the long and slender forms.

Undulating Membrane.—This, as in *Trypanosoma brucei*, is well developed, and thrown into many bold folds and undulations.

Flagellum.—The flagellum in the long and slender and intermediate forms is free. There is no free flagellum in the short and stumpy forms.

[illegible]

COMPARISON OF *Trypanosoma gambiense* WITH *Trypanosoma* *BRUCEI*.

On comparing the coloured plate of *Trypanosoma gambiense*, accompanying this paper (Plate 2), with that of *Trypanosoma brucei*,* it will be apparent that these two species of trypanosomes resemble each other very closely. There are the same long and slender, intermediate, and short and stumpy forms in both. The micronucleus is small and round, the nucleus oval or round, and the undulating membrane well developed. It may be concluded, then, that it is impossible to separate these two species by shape alone.

CHART II.—Chart giving Curves representing the Distribution, by Percentages, in respect to length of 1,000 Individuals of *Trypanosoma gambiense*, Uganda, and *Trypanosoma brucei*.



On comparing the curves representing the distribution by percentages in respect to length of 1,000 individuals of each species, some slight difference can be made out. It is seen that *Trypanosoma gambiense* lies more to the short end of the curve than *Trypanosoma brucei*. There are more non-flagellated forms in *Trypanosoma gambiense* than in *Trypanosoma brucei*: 38 per cent. in the former, 26 per cent. in the latter. It is doubtful, however, if this difference in the curve would always appear.

In the same way, if the 1,000 *Trypanosoma gambiense* are divided by length into short and stumpy (13 to 21 microns), intermediate (22 to 24 microns), and long and slender (25 microns and

* 'Roy. Soc. Proc.' B, vol. 83, Plate 2.

Trypanosoma gambiense.



Long & Slender.



Intermediate.



Short & Stumpy.

upwards), as was done in the case of *Trypanosoma brucei*, the following is the result:—

—	Short and stumpy.	Intermediate.	Long and slender.
	per cent.	per cent.	per cent.
<i>Trypanosoma gambiense</i> ...	51·2	23·1	25·7
<i>Trypanosoma brucei</i> ...	32·8	25·5	41·7

This shows the percentage of the intermediate to be much the same in the two species, whereas *Trypanosoma gambiense* is richer in short forms and poorer in long than *Trypanosoma brucei*.

Whether these slight differences are fundamental or only accidental it is impossible at present to say, but enough has been written to show that *Trypanosoma gambiense* and *Trypanosoma brucei* approach each other very closely in size and shape.

5.—A PRELIMINARY NOTE ON THE EXTRUSION OF GRANULES BY TRYPANOSOMES.*

By W. B. FRY, Captain R.A.M.C.

During some investigations carried out in the Wellcome Tropical Research Laboratories at the Gordon College, Khartoum, a phenomenon was noticed to occur which would seem to have some bearing on the life-history of the trypanosomata.

The observations were made whilst employing the dark ground method of illumination, and certain confirmatory evidence was obtained by using a modification of Levaditi's method of silver staining.

The trypanosome infection of animals referred to in this note was that caused by a strain known in the laboratories as Type I, *T. brucei* or *pecaudi*, a strain which our later conclusions lead us almost undoubtedly to regard as a variety of *T. brucei*.

It was found that at times during the course of an infection, certain of the trypanosomes extrude from their bodies granules which are thrown off apparently with considerable force, and then appear to possess a certain motility of their own in the blood.

The phenomenon has been observed both naturally and after drug treatment; its occurrence has been studied principally in the Jerboa, an animal in which the disease runs a chronic course, and it is considered that the extrusion of the granules bears some relation to the periodic disappearance of the trypanosomes from the circulating blood.

The granule is irregularly spherical in shape and of an apparently constant size, estimated at about 0·5 μ . At times a fine corkscrew-shaped filament was observed connected to these granules; this was seen sometimes immediately after extrusion; the length of the filament was estimated at four or five times the diameter of the granule. (See accompanying sketch, Plate 3.)

* Reprinted from the Proceedings of the Royal Society, B. Vol. 84.

It is believed that similar granules have been observed in the circulating blood in which trypanosomes were not to be found. Further, that the same granules have been identified in certain organs, viz., the lung in dogs, during the course of an infection.

The general appearance and character of the granules were in many ways very similar to those of an extrusion granule, which was subsequently observed by Dr. Andrew Balfour in the same laboratories as occurring in the spirochætosis of fowls, accounts of which have already been published. As far as possible the general fallacies due to dark ground illumination were avoided.

Further experiments as to the significance of this observation will be carried out later on in the year, but it is at present regarded as essentially of a vital and not of a degenerative nature.

6.—THE RELATION OF WILD ANIMALS TO TRYPANOSOMIASIS.

By Captain A. D. FRASER, R.A.M.C., and Dr. H. L. DUKE.

A.—EXAMINATION OF WILD ANIMALS WITH A VIEW OF ASCERTAINING WHETHER THEY ARE NATURALLY INFECTED WITH TRYPANOSOMIASIS.

I. *Animals frequenting the Lake-shore.*

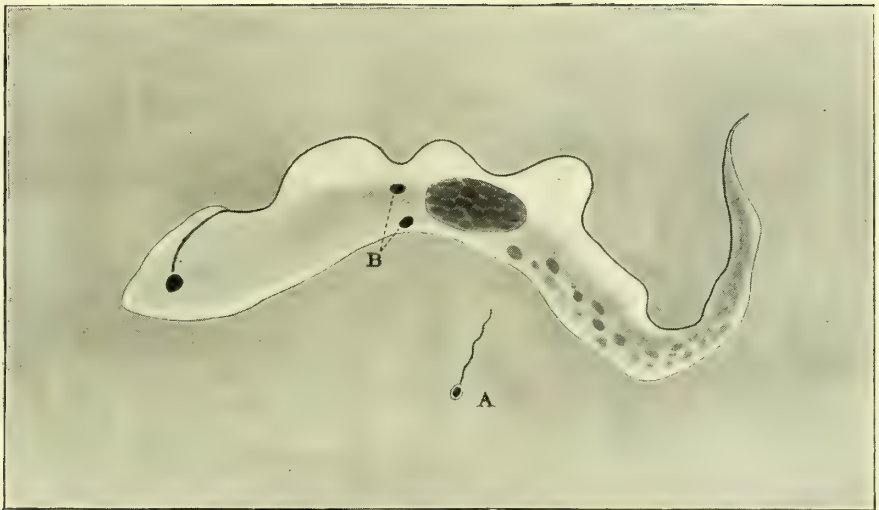
The work of the Sleeping Sickness Commission of the Royal Society, 1908-10, on the infectivity of the Lake-shore *Glossina palpalis*, and especially their experiments on artificially infected *Trypanosoma gambiense* antelope, made it appear probable that wild animals which frequent the shores of the Lake might be naturally infected with *Trypanosoma gambiense* and thus enable the flies to retain their infectivity for long after the removal of the inhabitants from the Lake-shore. It was, therefore, of the utmost importance to thoroughly examine the blood of all Lake-shore animals in order to ascertain if any evidence could be obtained in support of this.

A large drive was organised by Hamerton and Bateman. In August, 1910, large numbers of native hunters were got together and set to work near the Lake-shore. It may at once be said that the drive was not altogether a success. Many difficulties arose; food was difficult to obtain for so many people; animals were difficult to find; and it was not easy to arrange for the drives to take place where flies were most numerous. Although these hunters remained at work for nearly two months, their bag consisted of only four antelope and two bush pigs.

We, therefore, had to fall back upon week-end shoots.

The methods of examination adopted were:—

1. Microscopical examination of blood smears.
2. Injection of blood into susceptible animals.
3. Feeding laboratory-bred *Glossina palpalis* on the Lake-shore animal, and then, by feeding the flies on healthy susceptible animals and by dissecting the flies as they died, ascertaining whether the flies had been infected.



Trypanosome and granule, A, already flagellated, some time after extrusion, to show relative size. At B two granules are shown in the body of the trypanosome.



It was only found possible to carry out this last method of examination in the case of captured animals.

As susceptible animals for the injection of blood, monkeys and goats were, as a rule, both used. We were thus enabled to say whether the Lake-shore animals examined were naturally infected with trypanosomes, such as *T. vivax* and *uniforme*, to which monkeys are refractory.

Tables I. II. and III. show the results of examination of the Lake-shore antelope which were obtained. The interval in hours which elapsed after the death of the animal before the injection into the susceptible animals and the quantity of blood used are given.

Table I.—10 Lake-shore Waterbuck.

Date.	Experiment No.	Microscopical examination.	Interval in hours between death and injection.	Injection of blood into monkey.		Injection of blood into goat.		Buck, young or adult.
				Quantity in c.c.'s.	Result.	Quantity in c.c.'s.	Result.	
27.11.10	350	—	3	2	—	6	—	adult.
27.11.10	350	—	4	2	—	6	—	"
17.12.10	420	—	3	5	—	10	—	"
1.1.11	477	—	2	3	—	5	—	"
8.1.11	499	—	9	5	—	6	—	"
15.1.11	519	—	3½	3	—	5	—	"
15.1.11	520	—	5	3	—	5	—	"
29.1.11	548	—	1	4	—	10	—	"
6.2.11	590	—	19	3	—	5	—	"
18.3.11	697	—	3	3	—	5	—	"

Table II.—20 Lake-shore Bushbuck.

Date.	Experiment No.	Microscopical examination.	Interval in hours between death and injection.	Injection of blood into monkey.		Injection of blood into goat.		Buck, young or adult.
				Quantity in c.c.'s.	Result.	Quantity in c.c.'s.	Result.	
26.8.10	90	—	0	2	—	4	—	young.
7.9.10	123	—	0	2½	—	6	—	adult.
19.9.10	175	—	0	3½	—	5	—	young.
20.9.10	178	not examined	0	3	—	5	—	adult.
29.10.10	276	—	2	1	—	2	—	young.
20.11.10	339	++	1	5	—	10	—	adult.
26.11.10	351	not examined	0	3	—	4	—	} + { " young.
26.11.10	351	—	0	3	—	5	—	
7.1.11	496	"	2	3	—	6	—	adult.
4.2.11	589	—	½	3	—	5	—	"
12.2.11	596	—	3	3	—	6	—	"
12.2.11	619	—	3	3	—	6	—	young
18.2.11	617	not examined	1½	3	—	5	—	adult.
26.2.11	637	—	2	5	—	10	—	"
11.3.11	673	—	2	5	—	10	—	"
12.3.11	676	—	1½	4	—	8	—	"
12.3.11	680	—	1	2½	—	5	—	"
19.3.11	698	—	5	3	—	5	—	"
19.3.11	699	—	3	3	—	5	—	"
25.3.11	709	—	1	3	—	8	—	"

Table III.—2 Lake-shore Situtunga.

Date.	Experiment No.	Microscopical examination.	Interval in hours between death and injection.	Injection of blood into monkey.		Injection of blood into goat.		Young or adult.
				Quantity in c.c.'s.	Result.	Quantity in c.c.'s.	Result.	
6.9.10	134	—	0	5	—	7	—	young.
25.3.11	708	++	$\frac{1}{2}$	5	—	10	—	adult.

The 32 antelope examined were all shot or captured at the Lake-shore, the majority of them near where *Glossina palpalis* were collected for experiments which showed that the flies were still capable of conveying *Trypanosoma gambiense* to normal monkeys. None of the monkeys into which the blood of the antelope was injected developed trypanosomiasis. The same remark applies to goats, with the exception of one which had been injected with blood from two bushbuck. Ten days afterwards trypanosomes appeared in the goat's blood. In another paper this trypanosome is described and shown to be identical with *Trypanosoma uniforme*. In blood smears of bushbuck 339 and situtunga 708 what appeared to be the same trypanosome was found in fair numbers. The blood of these animals, however, did not infect the goats which were used for the injections. The goat which was injected with blood of bushbuck 339 was afterwards injected with blood of goat 352 infected with *T. uniforme*. The result of this injection was negative. It was later, however, infected by injecting blood of a *T. uniforme* infected ox. This suggests that a higher percentage of the antelope examined might have been shown to harbour this species of trypanosome if more susceptible animals, e.g., calves, had been employed.

Three 'cycle' experiments were carried out in which 27, 53, and 38 laboratory-bred *G. palpalis* were fed for seven days on bushbucks 90 and 123 and situtunga 134 respectively, and then until the 60th day of the experiments on normal monkeys. All these monkeys remained healthy. Ninety-six flies were dissected; all proved to be negative for flagellates.

The finding of a trypanosome, which appears to be carried by *Glossina palpalis*, in the blood of a situtunga is interesting. In the locality where the infected animal was shot, this species of antelope seldom leaves the large papyrus swamps, where it might be supposed they are little exposed to the bites of these flies.

The following note shows that *Glossina palpalis* do feed upon antelope. Bushbuck 673 was shot as it was approaching the Lake, and was still some 200 yards from the shore. When it was being stalked it appeared to be annoyed by flies. After the buck had been shot five or six *Glossina palpalis* were seen upon it. Some of the flies were observed to wander about the animal's back and side, probing with their probosces as they moved—apparently unable to reach the skin. They then settled down to feed on the less hairy parts, usually about the head and neck.

Tables IV. to VIII. show the other Lake-shore animals examined.

It will be noted that 22 hippopotami, 8 crocodiles, 12 monitors, 60 birds, and the animals in Table VIII. were examined without any of them showing trypanosomiasis.

Table IV.—22 Lake-shore Hippopotami.

Date.	Number of Experiment.	Microscopical Examination.	Interval in hours between death and injection.	Injection of Blood into Monkey.		Injection of Blood into Goat.	
				Quantity in c.c's.	Result.	Quantity in c.c's.	Result.
4.9.10	129	—	6	3	—	5	—
7.10.10	210	—	2	2½	—	5	—
8.10.10	220	—	7	2½	—	5	—
22.10.10	265	—	6	1½	—	5	—
23.10.10	"	—	4	1	—	3	—
25.10.10	"	—	7	1	—	3	—
26.10.10	"	—	3	1	—	3	—
26.10.10	"	—	3	1	—	3	—
29.10.10	"	—	4	1½	—	3	—
30.10.10	"	—	4	1½	—	2	—
1.12.10	291	—	2	1½	—	2	—
25.1.11	559	—	7	2	—	3	—
25.1.11	"	—	3½	2	—	3	—
28.1.11	"	—	3	2	—	5	—
3.2.11	593	—	7	2	—	3	—
3.2.11	"	—	5½	2	—	3	—
5.2.11	"	—	15	2	—	3	—
5.2.11	"	—	2	2	—	3	—
11.2.11	"	—	10	2	—	3	—
11.2.11	"	—	10	2	—	3	—
11.2.11	"	—	3	2	—	3	—
11.2.11	"	—	2	2	—	3	—

Table V.—8 Lake-shore Crocodiles.

Date.	Experiment No.	Microscopic Examination.	Interval in hours between death and injection.	Injection of Blood into Monkey.	
				Quantity in c.c's.	Result.
3.10.10	205	—	1	1	—
8.10.10	219	—	3	2½	—
11.10.10	"	—	1½	2½	—
		Hæmo-gregarines			
11.10.10	225	+	1	2	—
23.10.10	266	—	4	1½	—
		Hæmo-gregarines			
26.2.11	638	+	3	2	—
4.2.11	593	—	1½	3	—
10.2.11	593	—	2	3	—

Table VI.—12 Lake-shore Monitors.

Date.	Experi- ment No.	Micro- scopic Ex- amination.	Interval in hours between death and injection.	Injection of Blood into Monkey.		Injection of Blood into Goat.	
				Quantity in c.c.'s.	Result.	Quantity in c.c.'s.	Result.
3.10.10	204	—	1			2	—
7.10.10	221		2	2½	—		
26.10.10	235		0	1	—		
24.10.10	266		6	1	—		
27.10.10	"		4	2	—		
"	"		4	2	—		
"	"		4	2	—		
28.10.10	"		4	1	—		
28.10.10	"		4	1	—		
14.11.10	331		3	2½	Monkey died		
		Hæmo- gregarines					
26.2.11	638	+	3	2	—		
26.2.11	638	—	3	2	—		

Table VII.—60 Lake-shore Birds.

Date.	Experi- ment No.	Species.	No.	Microscopic examination.	Interval in hours between death and injection.	Blood injection into Monkey.	
						Quan- tity in c.c.'s.	Result.
22.8.10	108	Pigmy Goose	1	—			
4.9.10	130	Divers	2	—	1	2	—
28.9.10	192	Stork	1		0	2	—
28.9.10	196	Divers	7		2	3	}
		Cranes	2		2	1	
21.10.10	{ 267	Guinea Fowl	1		0	½	}
to		Egyptian Goose	1		1	1	
29.10.10	{ "	Divers	10		1	2½	}
4.11.10		Crane	1		1	1½	
to	{ 292						}
6.11.10		Divers	5		1	3	
				Halte- ridium			
11.11.10	515	Ducks	7	5	0	2½	}
	556	Crane	2	—	6	2	
	"	Fish Eagle	1	—	2	1	}
22.1.11	"	Divers	6	—	2	2½	
to	"	Egyptian Geese	2	—	8	2	}
28.1.11	"	Ibis	1	—	9	1	
	"	Stone Curlew	1	—	1½	1½	}
	"	Plover	1	—	1½	1½	
6.2.11	593	Egyptian Geese	2	—	3	}	3
	"	Divers	2	—	3		
8.2.11	"	Crane	1	Halte- ridium.	1	2	}
9.2.11	"	Egyptian Goose	1	—	2	}	
	"	Crane	1	—	2		
10.2.11	"	Egyptian Goose	1	—	0	1½	}

Table VIII.

Date.	Species.	Experiment No.	Microscopical examination.	Interval in hours between death and injection.	Injection into monkey.		Injection into goat.		Injection into white rat.	
					Quantity in c.c. s.	Result.	Quantity in c.c. s.	Result.	Quantity in c.c. s.	Result.
1910.										
Dec. 30	Man ...	473	—	0	$\frac{3}{4}$	—				
Aug. 27	Monkey ...	96	—							
" 22	Bush Pig ...	75	—	6	5	—	10	—		
Sept. 30	" ...	203	—	0			2	—		
Aug. 28	Edible Rat ...	100	—	?	2	—	2	—		
Oct. 31	" ...	259	—	0					$\frac{1}{2}$	—
Nov. 1	" ...	262	—	0					$\frac{1}{2}$	—
" 8	" ...	283	Not examined.	12					1	—
" 14	" ...	317	"	2	2	—				
" 22	" ...	317	"	0	1	—				
1911.										
Jan. 23	Otter ...	555	—	3	3	—				
" 24	" ...	555	—	$2\frac{1}{2}$	$1\frac{1}{2}$	—				
" 24	" ...	555	—	6	$1\frac{1}{2}$	—				
" 25	" ...	555	—	9	$1\frac{1}{2}$	—				
Feb. 6	" ...	593	—	$1\frac{1}{2}$	3	—				
" 7	" ...	593	—	$1\frac{1}{2}$	3	—				
" 10	Bat ...	593	—	1	2	—				

On the Island of Damba large numbers—at least 50—of *Glossina palpalis* were seen upon a monitor which was occupied in devouring the eggs in a crocodile's nest. On another occasion a Lake-shore fly was seen to feed to the full on a monitor which had just been shot.

On the other hand, it was extremely difficult to induce flies to feed on monitors in the laboratory.

The man, Experiment 473, was caught making a canoe on the Lake shore near Bukafu.

The following Table IX. summarises the results of the above examination of Lake-shore animals:—

Table IX.

Species of Animal.	Number examined.	Number showing Trypanosomes microscopically.	Number giving Positive result on injection.
Water Buck ...	10	—	—
Bush Buck ...	20	1	1
Situtunga ...	2	1	—
Hippopotami ...	22	—	—
Man ...	1	—	—
Monkey ...	1	—	—
Bush-Pig ...	2	—	—
Edible Rat ...	6	—	—
Otter ...	6	—	—
Bat ...	1	—	—
Crocodiles ...	8	—	—
Monitors ...	12	—	—
Birds ...	60	—	—

In connection with the positive injection Experiment 351, which resulted in the isolation of *T. uniforme*, it was thought advisable to carry out a control by sending a goat to live at Kibanga, where the injection had been performed, to ascertain whether the infection might be due to some source other than the buck's blood, *i.e.*, biting flies. This goat was kept for fourteen days at Kibanga, and then returned to Mpumu, where its blood was constantly examined for two months. No trypanosomes were ever detected. Further evidence on this point is the discovery of *T. uniforme* in stained films from bushbuck, Experiment 339, and situtunga 708, which render this control experiment unnecessary.

II. Animals obtained more than two miles from the Lake shore.

Table X. gives the results of examination of animals obtained more than two miles from the Lake shore:—

Table X.

Date.	Species.	Experiment No.	Microscopical examination.	Interval in hours after death.	Injection into monkey.		Injection into goat.		Injection into guinea-pig.	
					Quantity in c.c.'s.	Result.	Quantity in c.c.'s.	Result.	Quantity in c.c.'s.	Result.
1911.										
Jan. 27	Bushbuck ...	545	Not examined.	4½	5	—	5	—		
" 31	" ...	563	<i>T. ingens</i>	3	5	—	5	—		
Mar. 26	" ...	717	—	1½	1½	—	5	—		
" 26	" ...	717	—	1½	1½	—	5	—		
" 26	" ...	717	—	1½	1½	—	5	—		
1910.										
Aug. 30	Duiker ...	112	—	0	3	—				
Nov. 3	" ...	275	—	0	2½	—				
Dec. 6	Bush Pig ...	372	—	0	2½	—	2½	—		
Dec. 8	" ...	382	—	0	2	—	6	—		
1911.										
Feb. 24	" ...	634	—	0	3	—	5	—		
1910.										
Sept. 29	Mongoose ...	194	—	0					1	—
Oct. 19	Wild Cat ...	241	Not examined	0						—

Five bushbuck, two duiker, and three bushpig were examined. With the exception of a bushbuck which showed *T. ingens*, the results of the examination were all negative.

B. Experiments to ascertain if wild animals can be infected with *Trypanosoma gambiense*.

As a result of the work of Hamerton and Bateman on the question as to whether buck may not possibly act as a reservoir for

Trypanosoma gambiense, it was thought desirable to test other animals which frequent the Lake shore in order to ascertain whether or not they would give results similar to the antelope experiments. With this object in view, bushpig, edible rat, crocodile, monitor, frog and fowl were experimented with. With the one exception of the rat, none of the animals could be infected. Although the edible rat may occasionally, perhaps, be infected, no importance can be attached to the fact when the question of a reservoir is being considered.

Further details of the work are given below :—

(a) Bushpigs.

Experiment 33. Young Bushpig.

To ascertain if bushpig can be infected with T. gambiense.

1910.

- Aug. 30.—Injected subcutaneously 2 c.c. blood of *T. gambiense* infected monkey 2563.
- Oct. 3.—Injected subcutaneously $2\frac{1}{2}$ c.c. blood of *T. gambiense* infected monkey 86.
- „ 3-10.—Flies of positive *T. gambiense* transmission experiment 52 fed on pig.
- „ 7-11.—Flies of positive *T. gambiense* transmission experiment 97 fed on pig.
- „ 7-11.—Flies of positive *T. gambiense* transmission experiment 98 fed on pig.

Result.—Negative. Trypanosomes never appeared in the pig's blood. It was examined regularly until December 6. On September 14 and again on October 14 blood of the pig was injected into monkeys without causing them to become infected. Eighty-three laboratory-bred *Glossina palpalis* were fed on pig from September 12-15, and afterwards, September 17 to November 10, on a healthy monkey. This monkey did not become infected. Twenty-five of the flies were dissected and found to be negative for flagellates.

Experiment 372. Young Bushpig.

1910.

- Dec. 6.— $2\frac{1}{2}$ c.c. blood of pig injected into monkey 373; result, negative.
- „ 6-12.—Flies of positive *T. gambiense* transmission experiment 254 fed on pig.
- „ 16-19.—Flies of positive *T. gambiense* transmission experiment 268 fed on pig.

Result.—Negative. Trypanosomes were never found in the pig's blood, and blood of the pig when injected into a healthy monkey on January 6 did not cause an infection.

(b) Edible Rat.

Experiment 386. Edible Rat.

1910.

Dec. 8.—Injected subcutaneously $\frac{1}{2}$ c.c. blood of *T. gambiense* infected monkey 347.

„ 16.—*T. gambiense* present in rat's blood.

„ 20.—Rat died. Cause of death unknown.

Result.—Positive. On the 8th day, after having been injected with *T. gambiense*, trypanosomes appeared in the rat's blood.

(c) Crocodile.

Experiment 376. Young Crocodile.

1910.

Dec. 6.—Injected intraperitoneally 1 c.c. blood of *T. gambiense* infected monkey 297.

„ 19.—Crocodile killed; 1 c.c. heart blood injected into white rat 423.

Result.—Negative. Rat, Experiment 423, remained healthy.

(d) Monitor.

Experiment 235. Adult Monitor.

1910.

Oct. 13.—Monitor caught at Lake shore.

„ 14-19.—Nineteen laboratory-bred *G. palpalis*, Experiment 228, fed on Monitor.

Nov. 3.—Injected subcutaneously $1\frac{1}{2}$ c.c. blood of *T. gambiense* infected monkey 86.

„ 29-30.—Flies of positive *T. gambiense* transmission experiment 222 fed on monitor.

Dec. 1.—Monitor died. 1 c.c. blood injected into monkey 361.

Result.—Negative. Monkey 361 did not become infected. The monkey to which the flies of Experiment 228 were afterwards transferred remained healthy, and 14 of the flies used in the experiment were dissected and found negative for flagellates.

(e) Frog.

Experiment 277. Frog.

1910.

Nov. 3.—Injected subcutaneously $\frac{1}{2}$ c.c. blood of *T. gambiense* infected monkey 86.

„ 23.—Frog died. $\frac{1}{2}$ c.c. heart blood injected into guinea-pig 345.

Result.—Negative. Guinea-pig 345 was kept under observation for 50 days. It never showed trypanosomes.

(f) Fowls.

In the experiments shown in the following Table, with the exception of Experiments 103 and 104, which were monkey to monkey *T. gambiense* "cycles," all the flies used were originally fed upon *T. gambiense* infected antelope. These flies, though ultimately destined to infect monkeys, were at various periods

throughout the experiments nourished upon fowls. They thus afford evidence as to whether fowls can harbour *T. gambiense*.

Date.	Days of "cycle" on which flies fed upon fowl.	Experiment Number.	Number of flies used.	Did flies prove finally infective?	Result of inoculation of fowl's blood into monkey.
1910.					
Aug. 29	... (1) 26-33 (2) 37-45 (3) 50-59	} 103	107	No	—
" 29	... 26-33	104	175	"	—
Oct. 10	... (1) 14-29 (2) 34-42 (3) 46-54	} 212	101	Yes	—
" 10	... (1) 14-29 (2) 35-41	} 213	89	"	—
" 24	... (1) 11-26 (2) 30-37 (3) 42-46	} 245	20	"	—
" 24	... (1) 12-27 (2) 31-38 (3) 43-50 (4) 55-71	} 246	90	No	—
" 24	... (1) 11-26 (2) 30-32 (3) 36-42	} 247	72	Yes	—
" 24	... (1) 12-27 (2) 31-39 (3) 44-55 (4) 59-63	} 248	115	No	—
" 24	... (1) 12-27 (2) 31-38 (3) 44-52 (4) 58-62	} 249	168	"	—
Nov. 8	... (1) 13-28 (2) 33-39	} 284	72	Yes	—
" 14	... (1) 12-27 (2) 33-40	} 316	60	"	—
Dec. 26	... 14-26	460	50	"	—
" 26	... 14-26	461	49	No	—
" 26	... 14-26	462	43	"	—
" 26	... 14-26	463	25	"	—
1911.					
Jan. 6	... 11-29	484	54	Yes	—
" 7	... 9-30	490	96	No	—
" 12	... (1) 9-32 (2) 38-41	} 518	79	"	—
" 16	... (1) 10-29 (2) 36	} 522	45	"	—
" 25	... 8-28	540	36	"	—
" 30	... 10-27	551	40	"	—
" 30	... 10-27	552	70	Yes	—
" 30	... 10-27	553	54	No	—
Feb. 2	... 9-25	564	39	"	—
" 6	... 11-27	572	104	"	—
" 14	... 4-26	600	119	Yes	—
" 23	... 10-23	632	86	No	—

In Experiments 387 and 389 clean laboratory-bred *G. palpalis* were fed upon cocks 103, 104, 165, 212, 213, 245, 246, 247, 248,

249, 284, and 316. In these "cycle" experiments 151 flies were employed; 45 were dissected in Experiment 389, and 84 in Experiment 387. These all proved negative, while the monkeys upon which these two experiments were fed remained healthy.

Conclusions.—1. *Trypanosoma uniforme* was the only species of trypanosome obtained as the result of examination of wild animals, including 32 Lake-shore antelope.

2. The available evidence points to bushpig, crocodile, monitor, frog and fowls being refractory to *Trypanosoma gambiense*.

3. The edible rat, which is susceptible to *Trypanosoma gambiense*, can, by virtue of its habits, be of little importance in considering the question of a reservoir.

Mpummu, 10th April, 1911.

7.—THE DEVELOPMENT OF TRYPANOSOMES IN GLOSSINA PALPALIS.

By Capt. A. D. FRASER, R.A.M.C., and Dr. H. L. DUKE.

[Plates 4-15.]

All the flies used in the experiments described in this paper were hatched in the laboratory from pupæ collected on the Lake-shore. These were, as a rule, brought from the Island of Damba. Natives are sent out, and after a few hours' search on a sandy beach they returned with several thousand pupæ.

The temperature of the laboratory for the months during which the experiments were conducted is given in the following table:—

Table I.—Laboratory Temperature.

Month.	At 8 a.m.						At 4 p.m.					
	Dry Bulb.			Wet Bulb.			Dry Bulb.			Wet Bulb.		
	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
1910.												
August ...	66	70	62	—	—	—	75	80	66	—	—	—
September ...	66	71	64	—	—	—	77	80	72	—	—	—
October ...	68	71	65	—	—	—	76	85	68	—	—	—
November...	69	73	62	—	—	—	80	85	74	—	—	—
December...	68	74	62	—	—	—	80	84	73	—	—	—
1911.												
January ...	70	73	62	65	70	58	82	88	75	71	76	64
February ...	72	83	65	65	71	59	82	89	71	69	73	61
March ...	71	75	65	67	71	63	79	87	68	71	74	64

It seemed possible that the development in the fly might be favoured by keeping the cages at a slightly higher temperature. In a few of the experiments they were, therefore, placed over a basin of water in the incubator, which was set so that the temperature never fell below 25° C. It will be seen in the details which of the experiments were so dealt with. It was found that the flies died early in the experiment, and no conclusion could be arrived at.

The relative humidity in the laboratory usually ranged between 50 and 80 per cent.

A.—THE DEVELOPMENT OF *TRYPANOSOMA GAMBIENSE* IN *GLOSSINA PALPALIS*.

The experiments mentioned below refer only to those in which the infected animals first fed upon by the flies were monkeys. In a later paper experiments relating to the transmission of the same species of trypanosome by feeding flies on infected antelope will be considered. The nature of the infection in positive flies of these latter experiments, and the morphology of the flagellates found in the flies are, however, included here.

The following table shows the number of flies employed in each experiment, (1) at its commencement, (2) on the 30th day, and (3) on the 50th day, the number of days the flies were fed on the infected monkey, the number of days after the first infected feed which elapsed before the flies became infective, and the length of the experiment in days. The minus sign indicates that the flies failed to infect the healthy monkey.

Table II.—Development of *Trypanosoma Gambiense* in *Glossina palpalis*.

Experiment.	Number of flies			Number of days		
	on 1st day.	on 30th day.	on 50th day.	fed on.	before flies be- came infective.	experiment lasted.
4A.	107	48	30	2	—	70
4B.	175	7	—	2	—	36
49	?	?	26	2	—	72
50	?	?	13	2	32-35	62
358	29	16	12	1	—	51
359	22	11	8	1	—	51
360	22	4	2	1	—	51

Only one of these seven experiments succeeded in transferring the infection from the infected to the healthy monkey. The exact day on which the flies became infective is uncertain, as the monkey which was infected was examined only twice a week.

The details of the experiments are as follows:—

To ascertain if a development of *Trypanosoma gambiense* occurs in *Glossina palpalis*.

Experiment 4A.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Aug. 3	—	107 flies fed on infected monkey, 2554.			Aug. 3, Trypanosomes present in monkey 2554's blood.
" 4	1	" "			
" 5	2	Flies starved 48 hours.			
" 6-27	3-24	Flies fed on healthy monkey, 13.			
" 28	25	Flies starved 48 hours.			
" 29- Sept. 5.	26-33	Flies fed on healthy fowl, 103.			Sept. 26, monkey 13 died.
Sept. 6-8	34-36	Flies fed on healthy monkey, 13.			
" 9-17	37-45	Flies fed on healthy fowl, 103.			
" 18	46	Flies starved 48 hours.			
" 19-21	47-49	Flies fed on healthy monkey, 13.		—	
" 22- Oct. 1.	50-59	Flies fed on healthy fowl, 103.			Oct. 12, 18 remaining flies dissected.
Oct. 2	60	Flies starved 48 hours.			
" 3-8	61-66	Flies fed on healthy monkey, 200.		—	
" 9-12	67-70	Flies starved.			

Remarks.—The procedure adopted here of feeding the flies alternately on a healthy monkey and fowl was intended to exclude the possibility of the flies ingesting trypanosomes during the incubation period in the healthy monkey in the event of the experiment becoming a positive one. Monkey 13 died on September 26th apparently from anaemia caused by the loss of blood. Monkey 200 remained healthy. As the flies died, they were dissected. Six flies containing flagellates were found—all of them during the first 22 days of the experiment. The positive fly, which died on the 22nd day, was injected intraperitoneally into a white rat. This rat did not become infected.

Experiment 4B.

To ascertain if temperature has an influence on the development of *Trypanosoma gambiense* in *Glossina palpalis*.

Remarks.—The flies were kept in the incubator set at a minimum temperature of 25° C. The procedure was otherwise the same as in Experiment 4A, which served as a control. Only 7 flies were alive on the 30th day, and on the 36th day of the

experiment the last fly died. The healthy monkey 14 on which the flies had been fed did not become infected. Twenty flies on dissection were found to contain flagellates—one on the 30th day of the experiment, the others on or before the 17th day. The intestinal contents of positive flies dying on the 13th, 15th, and 17th days of the experiment were injected intraperitoneally into white rats, none of which became infected.

Experiment 49.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Aug. 16-17	1	Flies fed on infected monkey, 2562.			Trypanosomes numerous in monkey, 2562.
„ 18	2	Starved 48 hours.			
„ 19-22	3-6	Flies fed on healthy fowl.			
„ 23-24	7-8	Flies fed on healthy monkey, 49.			Aug. 25, monkey 49 died.
„ 25- Oct. 27	9-72	Flies fed on healthy monkey, 49A.		—	Oct. 27, 26 remaining flies dissected.

Remarks.—The 26 remaining flies on dissection all proved to be negative for flagellates.

Experiment 50.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Aug. 16-17	1	Flies fed on infected monkey, 21.			Trypanosomes numerous in monkey, 21.
„ 18	2	Starved 48 hours.			
„ 19-22	3-6	Flies fed on healthy fowl.			
„ 23- Oct. 15	7-60	Flies fed on healthy monkey, 50.	+		Oct. 17, remaining 13 flies dissected.
Oct. 15-17	61-62	Flies starved.			

Remarks.—The flies of this experiment became infective between the 32nd and 35th days after the first infected feed. In order to collect the clear fluid dropped by the flies after a feed, they were kept on the monkey until the 60th day. The remaining flies were all negative for flagellates.

Experiments 358, 359, 360.

Remarks.—The three cages were fed on December 1st on three different infected monkeys, in each of whose blood there were numerous trypanosomes. All were then starved for 144 hours,

and then, until the 50th day, were fed on the same healthy monkey—381. This monkey did not become infected. No fly containing flagellates was found after the 27th day in any of the experiments. These experiments were made with the view of ascertaining if the want of food for several days would influence the development of flagellates in the alimentary canal of the fly.

Note.—Dr. van Someren had charge of Experiments 49 and 50 until September 6th.

The following table gives the number of flies dissected in each of the experiments detailed above, the number of positive flies found, and the result of injection of positive flies:—

Table III.—Fly Dissections of the Above Experiments.

Experi- ment No.	—	Days of Experiment.									Totals.
		1-5	5-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	
4A	Dissected	7	13	32	8	13	3	11	19	—	106
	Positive	—	4	1	1	—	—	—	—	—	6
	Injection	—	—	—	—	—	—	—	—	—	—
4B	Dissected	10	26	120	9	5	—	—	—	—	170
	Positive	5	11	3	1	—	—	—	—	—	20
	Injection	—	—	—	—	—	—	—	—	—	—
49	Dissected	—	—	—	—	—	—	—	—	26	26
	Positive	—	—	—	—	—	—	—	—	—	—
	Injection	—	—	—	—	—	—	—	—	—	—
50	Dissected	—	—	—	—	—	—	—	13	—	13
	Positive	—	—	—	—	—	—	—	—	—	—
	Injection	—	—	—	—	—	—	—	—	—	—
358	Dissected	4	3	5	—	2	2	11	—	—	27
	Positive	1	—	1	—	—	—	—	—	—	2
	Injection	—	—	—	—	—	—	—	—	—	—
359	Dissected	—	3	3	4	1	2	8	—	—	21
	Positive	—	—	—	2	—	—	—	—	—	2
	Injection	—	—	—	—	—	—	—	—	—	—
360	Dissected	4	9	3	1	1	1	2	—	—	21
	Positive	2	—	—	—	—	—	—	—	—	2
	Injection	—	—	—	—	—	—	—	—	—	—
Totals	Dissected	25	54	163	22	22	8	32	32	26	384
	Positive	8	15	5	4	—	—	—	—	—	32

It is seen that while 32 infected flies were found during the first 30 days of these experiments none were present among the flies which were dissected later than the 30th day. It is to be noted that in the one experiment—No. 50—which successfully transmitted the infection from the infected to the healthy monkey, the flies remaining on the last day were the only ones which were dissected.

Site of Infection in Infected Flies.

In all the positive flies found infected with this species of trypanosome the gut, especially the mid-gut, was the chief site of infection with flagellates. The thoracic intestine was often free, and, when infected, the flagellates usually occurred at intervals along its course congregated into dense masses. In the

majority of flies, especially among those found infected during the earlier stages of the experiments, the proventriculus was negative. On a few occasions flagellates were seen in the salivary glands. Infection of the proboscis was never observed. On one occasion the juice obtained from the proboscis by irritating the living fly was found to contain a flagellate. This occurred during the final dissection of Experiment 190, and the gut and proventriculus of the fly in question were found swarming with trypanosomes. The single trypanosome seen was small and showed slow undulatory movements. It was injected into a white rat with negative result.

Morphology of Flagellates in the Fly.—Stained, show very marked polymorphism—from small round forms suggestive of Leishman-bodies to the long attenuated type found in the proventriculus.—

(a) Gut:—Types 1, 2, 3, 4, 6, 9, Plate 4, are most common. Types 11, 17, 19, Plate 4, are present in fair numbers, and together with types 10, 11, 13, 16, which occur especially in earlier flies, appear to constitute a developmental series. No forms with a free flagellum were seen.

(b) Proventriculus:—Types 2, 3, 10, 11, Plate 6, are peculiar to the proventriculus, the flagellar end being greatly drawn-out and terminating in a well-marked free flagellum. In other flies these slender forms are not found, the majority being of types 15, 16, 17, Plate 6.

(c) Proboscis:—No proboscis infection was seen.

In a 38-day fly of Experiment 469 which fed originally upon reedbuck 2357, a remarkable type of flagellate was found in considerable numbers. This is shown in Plate 7. The parasites were present only in the intestine, and resemble Crithidia in structure, though the undulating membrane is poorly developed or apparently absent. In the fresh state they are at once distinguished from trypanosomes by their outline and by the rigidity of the posterior portion of the body. Movement is fairly rapid, and they frequently bore through masses of debris with the flagellum in advance. The flagellum is long, gradually tapering from a thickened base, and shows a steady undulating movement in which only the extreme anterior end of the body participates. The protoplasm is vacuolated in the living animal. In stained preparations, as will be seen in the plate, the two nuclei are close together, the micro-nucleus being relatively large and at times causing a slight projection on the side of the body.

Besides the elongated type shown in figs. 1, 2, 3, &c., rounded examples were met with as shown in figs. 4-8 without any undulating membrane. Fig. 9 shows a form with no trace of flagellum or undulating membrane. Binary fission was observed in the living animal. With reference to the nature of this flagellate, it may be noted that it is quite unique, as regards its morphology, from anything we have previously found in *Glossina palpalis*. The flies of this experiment fed from 11th to 29th days on a cock whose blood appeared negative to flagellates both by microscopical examination and by injection into a monkey.

B.—THE DEVELOPMENT OF *TRYPANOSOMA BRUCEI* IN *GLOSSINA PALPALIS*.

The strain of *Trypanosoma brucei*—monkey experiment 2566—left at Mpumu by Hamerton and Bateman was employed in these experiments.

The manner in which the strain was kept up is shown in the following table:—

Table IV.—Showing how the *T. brucei* strain was kept up.

Date.	No. of Experiment.	Source of Virus.	Incubation Period in days.	Duration of Disease in days.*	Remarks.
CALF.					
1910. Nov. 3	269	Fly infection from Monkey 2566.	?	—	See experiment 155. Calf alive after 150 days.
MONKEY.					
1910. June 18	2566	?	?	87	Died of <i>T. brucei</i> .
Sept. 20	44	Fly infection from Monkey 2566.	?	20	See experiment 42. Died of <i>T. brucei</i> .
" 13	159	Monkey 2566 ...	—	—	Under observation 108 days.
Oct. 21	162	Fly infection from Monkey 2566.	?	42	See experiment 155. Died of <i>T. brucei</i> .
Dec. 2	367	Monkey 162 ...	26	31	Died of <i>T. brucei</i> .
1911. Jan. 31	472	Fly infection from Monkey 367.	?	16	" "
GUINEA-PIG.					
1910. Sept. 13	160	Monkey 2566 ...	—	—	Under observation 60 days.
Oct. 5	206	Rat 185 ...	—	—	Under observation 58 days.
" 5	207	" 186 ...	—	—	Under observation 58 days.
" 25	255	" 186 ...	7	43	Died of <i>T. brucei</i> .
Dec. 7	380	Guinea-pig 255 ...	9	81	" "
1911. Jan. 7	489	" " 380 ...	23	—	Still alive after 81 days.
" 24	537	" " 380 ...	7	42	Died of <i>T. brucei</i> .
Mar. 14	677	" " 537 ...	10	—	Still alive after 15 days.
WHITE RAT.					
1910. Sept. 26	185	Fly injection from Monkey 2566.	4	38	See experiment 42. Died of <i>T. brucei</i> .
" 26	186	Fly injection from Monkey 2566.	7	27	See experiment 42. Died of <i>T. brucei</i> .

* Note.—Duration of disease includes incubation period when this is known.

Table V.—Development of *Trypanosoma brucei* in *Glossina palpalis*.

Experiment.	Number of flies			Number of days		
	on 1st day.	on 30th day.	on 60th day.	fed on.	before flies became infective.	experiment lasted.
42	116	100	45	2	32	64
43	70	16	—	2	—	45
155	59	30	19	3	33	62
156	70	63	45	3	—	79
273	19	17	12	1	—	64
470	75	57	—	2	26	36

Three of the six experiments were successful.

Here follow the experiments in detail.

Experiment 42.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive	Negative.	
1910. Aug. 12-13	1	116 flies fed on infected monkey, Experiment 2566.	+		Trypanosomes numerous in blood of 2566.
" 14	2	Starved 48 hours.			
" 15 to Sept. 20	3-39	Fed on healthy monkey, 44.			
Sept. 21 to Oct. 13	40-62	Fed on calf, 181.		—	
Oct. 14-15	63-64	Starved.			Oct 15, remaining 41 flies dissected.

Remarks.—The healthy monkey was infected on the 32nd day of the experiment. The calf which was fed upon from the 40th day onwards remained healthy. The positive fly which seemed to be the cause of the infection in the monkey died on the 45th day—the 5th day after the cage was first put on the calf. This fly may thus not have fed on the calf. A drop of citrated intestinal contents of this positive fly was injected intraperitoneally into a white rat. This rat became infected with *T. brucei*. The salivary glands of the same fly were injected intraperitoneally into another white rat which also became infected. The gut contents of an 8-day fly and the gut and salivary glands of a 36-day fly were injected intraperitoneally into white rats with negative results.

Experiment 43.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Aug. 12-13	1	70 flies fed on infected monkey, 2566.			Kept in incubator. Experiment 42 serves as control.
" 14	2	Starved 48 hours.			
" 15- Sept. 26.	3-45	Fed on healthy monkey. Experiment 45.		—	Sept. 26. Remaining 2 flies dissected.

Remarks.—In this experiment the healthy monkey did not become infected. Positive flies were found on the 6th, 7th, 11th, 26th, and 27th days. The intestinal contents of each of these flies and the salivary glands of the 26- and 27-day flies were injected into rats or guinea-pigs, none of which became infected.

Experiment 155.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Sept. 10-13	1-3	59 flies fed on infected monkey, 2566.			Trypanosomes numerous in 2566. Cage kept in incubator.
" 14	4	Starved 48 hours.			
" 15 to Oct. 20.	5-40	Fed on healthy monkey, 162.	+		
Oct. 21	41	Starved 48 hours.			
" 22 to Nov. 2.	42-53	Fed on healthy calf, 269.	+		
Nov. 3-4	54-55	Starved 72 hours.			
" 5-9	56-60	Fed on healthy bush buck, 660.		—	Nov. 11. Remaining 19 flies dissected.
" 10-11	61-62	Starved.			

Remarks.—The flies of this experiment became infected on the 33rd day. They retained their infectivity when transferred on the 42nd day to a healthy calf. From this day onwards, although all the flies were dissected as they died, none were found to contain flagellates. Either the infection in the fly responsible for the positive results of this experiment escaped detection on dissection, or the fly had in some way cleaned itself. It should be mentioned that on the 26th day of the experiment two flies died which were not dissected. As an additional precaution the gut contents of the remaining 19 flies were pooled and injected subcutaneously into a monkey; the result was negative.

Experiment 156.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Sept. 10-13	1-3	70 flies fed on infected monkey, 2566.			To serve as control to experiment 155. Not kept in incubator. Nov. 28. Remaining 33 flies dissected.
" 14	4	Starved 48 hours.			
" 15- Nov. 26	5-77	Fed on healthy monkey, 163.		—	
Nov. 27-28	78-79	Starved.			

Remarks.—This experiment was negative. Monkey 163 remained healthy and no positive flies were found throughout.

Experiment 273.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Nov. 3	—	19 flies fed on calf, 269.			Nov. 3. Trypanosomes present for first time in calf.
" 4-9	1-6	Starved 168 hours.			Nov. 17. Monkey 305 died.
" 10-16	7-13	Fed on healthy monkey, 305.		—	
" 17	14	Starved 48 hours.			
" 18-Jan. 4 (1911).	15-62	Fed on healthy monkey. Experiment 330.		—	Jan. 6. Remaining 12 flies dissected.
1911. Jan. 5-6	63-64	Starved.			

Remarks.—This experiment was also negative, both as regards monkeys and flies.

Experiment 470.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Dec. 28-29	1	75 flies fed on monkey, 367.			Extraordinary numbers of Trypanosomes in blood of 367.
" 30 Dec. 31-Jan. 31 (1911).	2 3-34	Starved 48 hours. Fed on healthy monkey, 472.	+		
1911. Feb. 1-2	35-36	Starved.			Feb. 2. Remaining 54 flies dissected.

Remarks.—The result here was positive. The monkey was infected on the 26th day of the experiment and eight of the remaining 54 flies were found to be positive on dissection. The feeding

of the flies on the infecting animal was commenced on the first day on which trypanosomes were found in the monkey and when they were present in quite exceptional numbers.

Table VI.
Fly Dissections of the above experiments.

Experiment No.	—	Days of Experiment.									Totals.
		1-5	5-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	
42	Dissected	3	6	3	3	12	31	14	42	—	114
	Positive	1	1	—	—	1	1	—	—	—	4
	Injection	—	—	—	—	—	+	—	—	—	—
43	Dissected	3	10	22	18	12	4	—	—	—	69
	Positive	—	2	1	2	—	—	—	—	—	5
	Injection	—	—	—	—	—	—	—	—	—	—
155	Dissected	—	—	5	8	2	6	3	19	—	43
	Positive	—	—	—	—	—	—	—	—	—	—
	Injection	—	—	—	—	—	—	—	—	—	—
156	Dissected	—	—	6	—	2	6	8	5	39	66
	Positive	—	—	—	—	—	—	—	—	—	—
	Injection	—	—	—	—	—	—	—	—	—	—
273	Dissected	—	—	—	1	—	—	5	12	—	18
	Positive	—	—	—	—	—	—	—	—	—	—
	Injection	—	—	—	—	—	—	—	—	—	—
470	Dissected	9	6	—	3	56	—	—	—	—	74
	Positive	1	—	—	—	8	—	—	—	—	9
	Injection	—	—	—	—	—	—	—	—	—	—
Totals	Dissected	15	22	36	33	84	47	30	78	39	384
	Positive	2	3	1	2	9	1	—	—	—	18

Remarks.—It is to be noted from the above table that those experiments (42 and 470) in which a positive fly was found from the 30th day onwards were positive in result.

None of the positive flies of Experiment 470 were injected.

Site of Infection in Infected Flies.

The nature of infection in the fly appears to correspond closely with what has already been described for *Trypanosoma gambiense*. Here again the proboscis has never been found to contain flagellates, while the gut is constantly infected. Large rosettes occupying one-third of the field, with low magnifications, were found in several flies dissected during the earlier days of the experiments. Infection of the proventriculus appears to be on similar lines to that in *T. gambiense*. Flagellates were found on two occasions in the salivary glands, and in one case—a 45-day fly—proved positive on inoculation.

Morphology of Flagellates in the Fly—stained.

Plates 8 and 9 show the different types of flagellates in flies infected with *T. brucei*. It will be seen that the types are

fairly uniform. The trypanosome form is usually retained. The micronucleus is small and rounded, and is usually situated fairly near the antflagellar extremity, which is often drawn out and pointed.

Plates 9, 10 and 11 show drawings of the *T. brucei* strain employed in the above experiments.

C.—THE DEVELOPMENT OF *TRYPANOSOMA PECORUM* IN *GLOSSINA PALPALIS*.

Two monkeys—Experiments 2579 and 2589—infected with this trypanosome were left by Hamerton and Bateman.

Table VII.—Showing how the *T. pecorum* strain was kept up.

Date.	Experiment No.	Source of virus.	Incubation period in days.	Duration of disease in days.*	Remarks.
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CATTLE.

1911.					
Jan. 10	509	Monkey 174 ...	11	35	Died of <i>T. pecorum</i> .
„ 18	609	„ 152 ...	13	—	Still alive after 71 days.

MONKEY.

1910.					
June 27	2579	?	46	150	Died of <i>T. pecorum</i> .
?	2589	?	?	?	
Sept. 8	152	Guinea-pig 78 ...	8	—	Still "alive after" 203 days.
Dec. 19	174	Fly infection from Monkey, 152.	?	35	Died of <i>T. pecorum</i> .

GUINEA-PIG.

Aug. 23	78	Monkey 2589 ...	14	16	Died of <i>T. pecorum</i> .
Nov. 23	344	„ 2579 ...	—	—	Under observation 51 days.
1911.					
Feb. 18	610	„ 152 ...	—	—	Under observation 37 days.

*Note.—Duration of disease includes incubation period when this is known.

Table VIII.—Development of *Trypanosoma pecorum* in *Glossina palpalis*.

Experiment.	Number of flies			Number of days		
	on 1st day.	on 30th day.	on 60th day.	fed on	before flies became infective.	experiment lasted.
66	59	39	23	2	—	77
67	77	19	3	2	—	77
167	101	89	47	2	—	95
168	86	65	47	2	85	108
343	60	49	25	2	—	105
349	59	53	23	2	—	94
426	53	15	2	2	—	63

Of the seven experiments carried out only one was successful.

Experiment 66.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Aug. 19-20	1	59 flies fed on infected monkey, 2589.			Trypanosomes present in monkey 2589's blood.
" 21	2	Starved 48 hours.			
" 22- Nov. 2.	3-75	Fed on healthy monkey, 73.		—	Nov. 4. 17 remaining flies dissected.
Nov. 3-4	76-77	Flies starved.			

Remarks.—Monkey 73 remained healthy. The only fly which was found to contain flagellates was one which was dissected three days after it had fed on the infected monkey. The gut contents of the 17 remaining flies were pooled and injected subcutaneously into a healthy guinea-pig. The result of this injection was negative.

Experiment 67.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Aug. 19-20	1	77 flies fed on infected monkey, 2589.			Flies kept in incubator. Experiment 66 serves as control.
" 21	2	Starved 48 hours.			Sept. 19. Cage taken from incubator and kept at laboratory temperature.
" 22- Nov. 2.	3-75	Fed on healthy monkey, 74.		—	Nov. 4. 2 remaining dissected.
Nov. 3-4	76-77	Starved.			

Remarks.—The healthy monkey did not become infected. Positive flies were found on the 4th, 13th, 14th, 29th, 43rd, and

77th days of the experiment. Those found on and after the 13th day were injected intraperitoneally into white rats, all of which remained negative.

Experiment 167.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1910. Sept. 16-17	1	101 flies fed on infected monkey, 152.			Trypanosomes present in monkey 152 for first time.
" 18	2	Starved for 48 hours.			
" 19-	3-67	Fed on healthy monkey, 173.			
Nov. 22.					
Nov. 23-28	68-73	Fed on healthy goat, 346.		—	
" 29-	74-92	Fed on healthy monkey, 173.		—	
Dec. 17.					
Dec. 18-20	93-95	Starved.			Dec. 20, 3 remaining flies dissected.

Remarks.—On the 5th, 59th, and 61st days of the experiment positive flies were found. The proboscis, proventriculus, and intestinal contents of the 59-day fly and the proboscis and proventriculus of the 61-day fly were injected subcutaneously into a healthy calf. This calf did not become infected. The gut of the 61 day fly was injected subcutaneously into white rat. The result of this injection was also negative.

Experiment 168.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1910. Sept. 16-17	1	86 flies fed on infected monkey, 152.			Flies kept in incubator.
" 18	2	Starved 48 hours.			Experiment 167
" 19-	3-85	Fed on healthy monkey, 174.	+		serves as control.
Dec. 10.					Nov. 16, cage re-
Dec. 11	86	Starved 48 hours.			moved from in-
" 12-17	87-92	Fed on healthy monkey, 173.		—	cubator and kept
" 18-19	93-94	Starved 72 hours.			at room tempera-
" 20-22	95-97	Fed on healthy monkey, 427.		—	ture.
" 23-31	98-106	Fed on healthy monkey, 439.		—	Nov. 30, cage re-
					placed in incuba-
					tor.
1911. Jan. 1	107	Flies starved.			Jan. 2, 4 remain-
					ing flies dissected.

Remarks.—*T. pecorum* appeared in the blood of monkey 174 on December 19. A fly which died on December 21st—the 96 day of

the experiment—showed a continuous infection with flagellates of the alimentary canal, extending from the proboscis to the point where the Malpighian tubes join the gut. Portions of the proventriculus and of the gut were injected subcutaneously into white rats. The remainder of the proventriculus and gut along with the proboscis was injected subcutaneously into a calf. The results of these injections were all negative. With the exception of two, dying on the 94th day, all flies after the 70th day were dissected and found negative. If the one positive fly found in this experiment infected monkey 174, it must afterwards have become non-infective, as during the last 11 days of its life it had access to monkeys 173 and 427 upon one at least of which it must have fed.

Experiment 343.

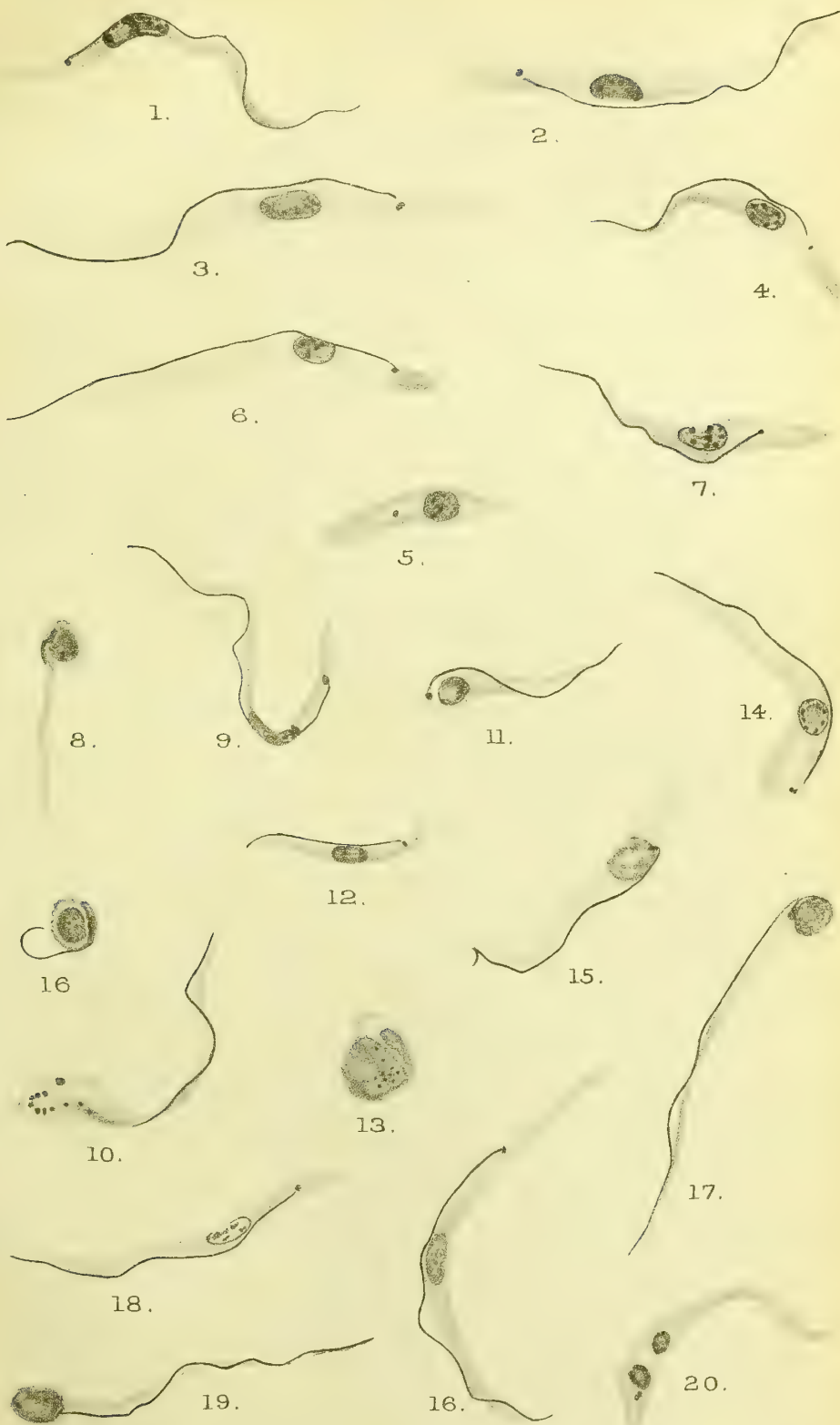
Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Nov. 21-22	1	60 flies fed on infected monkey, 2579.			No Trypanosomes present in monkey 2579's blood. Mar. 6, 4 remaining flies dissected.
„ 23	2	Starved 48 hours.			
„ 24-	3-103	Fed on healthy monkey, 348.		—	
1911, Mar. 4. Mar. 5-6	104-105	Starved.			

Remarks.—No positive flies were found in this experiment.

Experiment 349.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1910. Nov. 25-26	1	59 flies fed on infected monkey, 152.			Trypanosomes fairly numerous in monkey 152. Feb. 27, 3 remaining flies dissected.
„ 27-28	2-3	Starved 72 hours.			
„ 29-	4-92	Fed on healthy monkey, 354.		—	
1911, Feb. 25. Feb. 26-27	93-94	Flies starved.			

Remarks.—Positive flies were found on the 10th(2), 33rd, 59th, and 74th days of the experiment. The proboscis and intestinal contents of the flies found on the 59th and 74th days were injected subcutaneously into white rats with negative results.



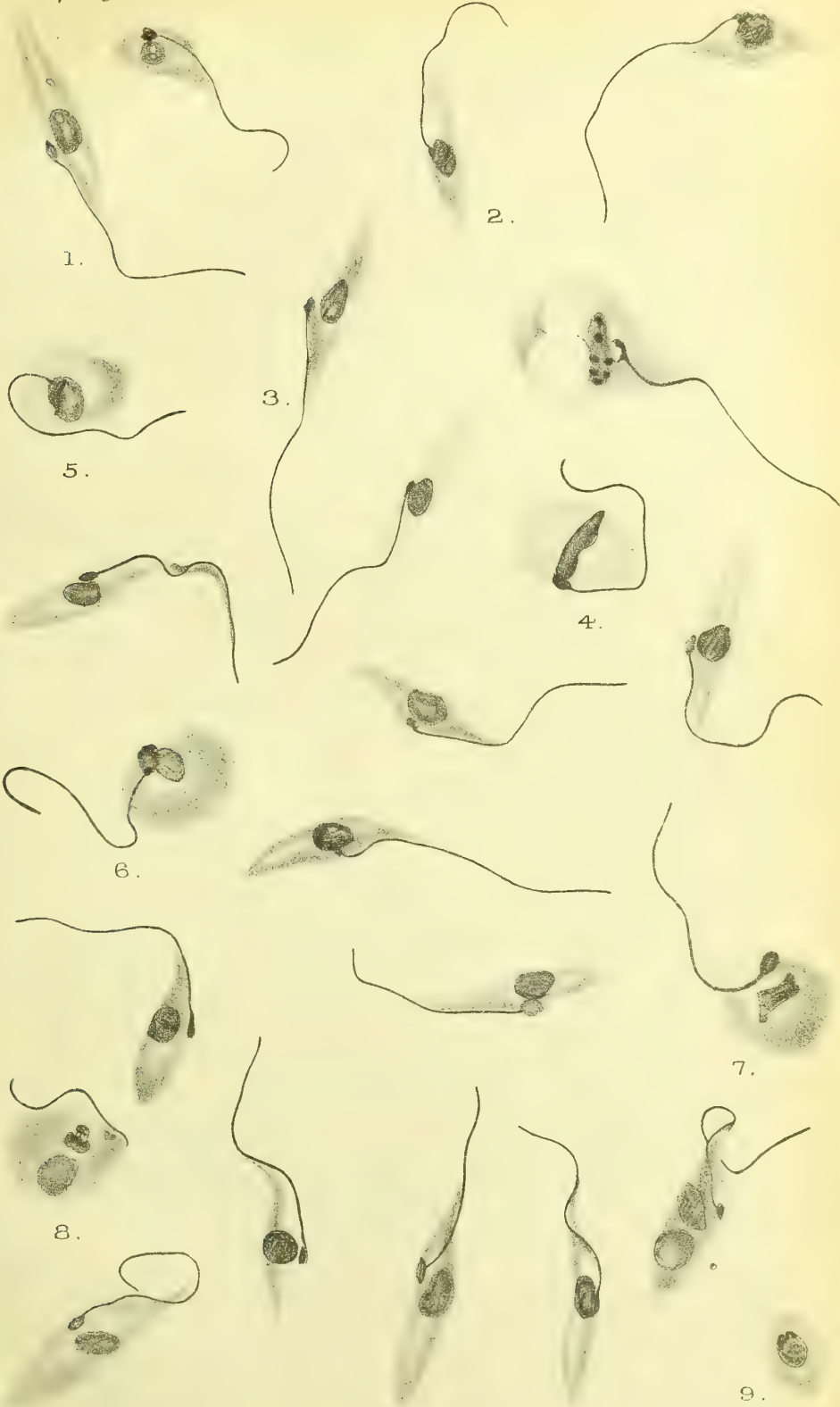




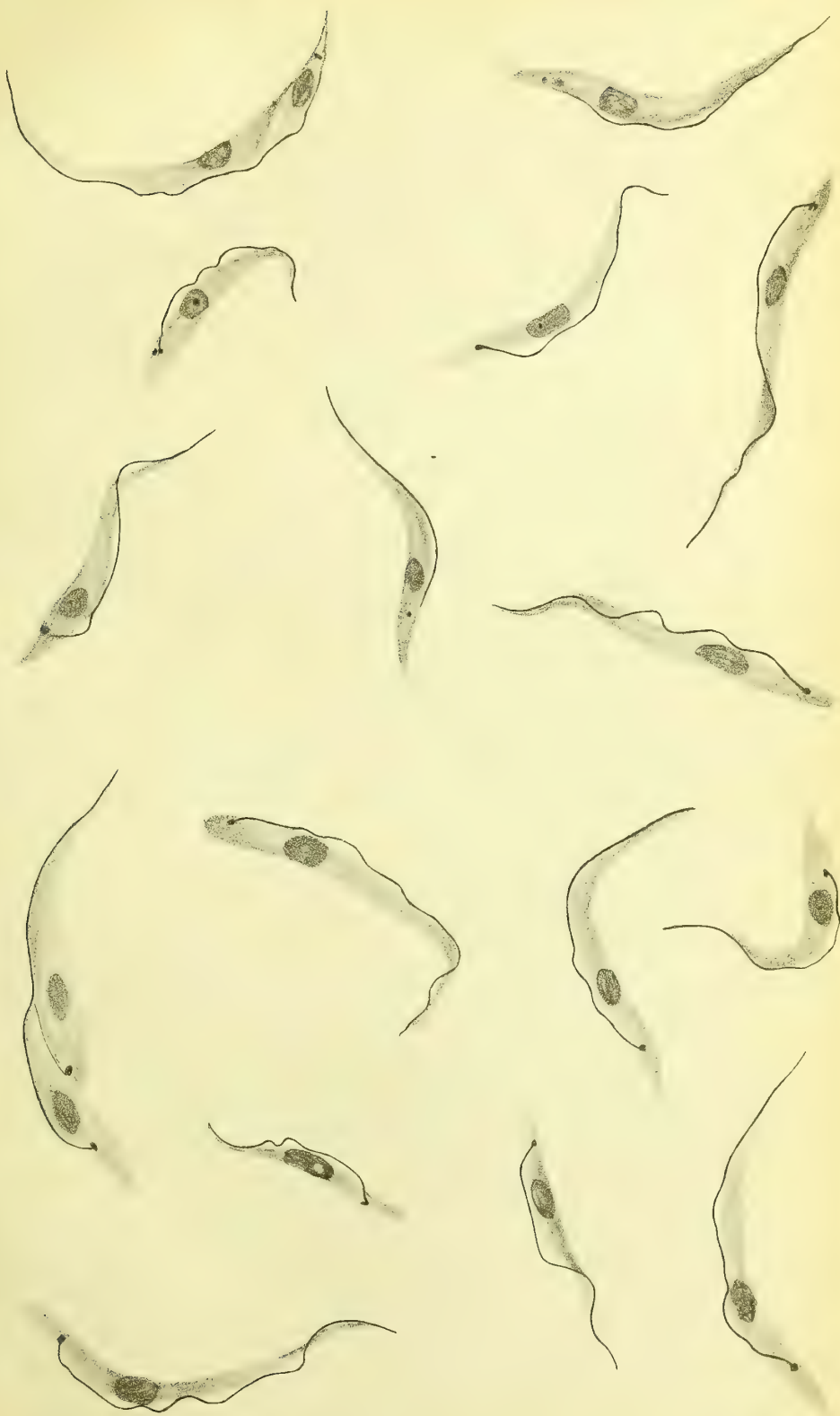








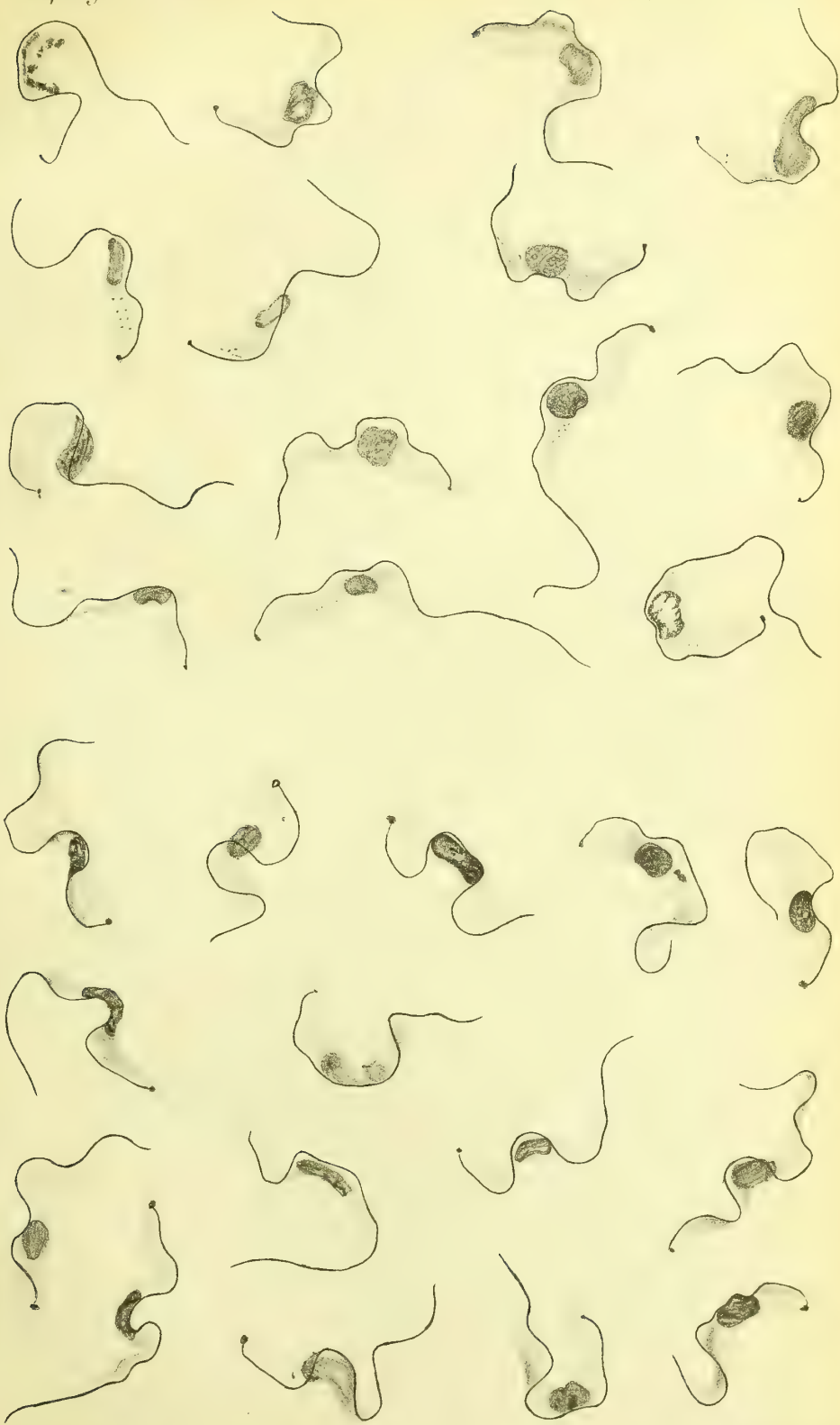


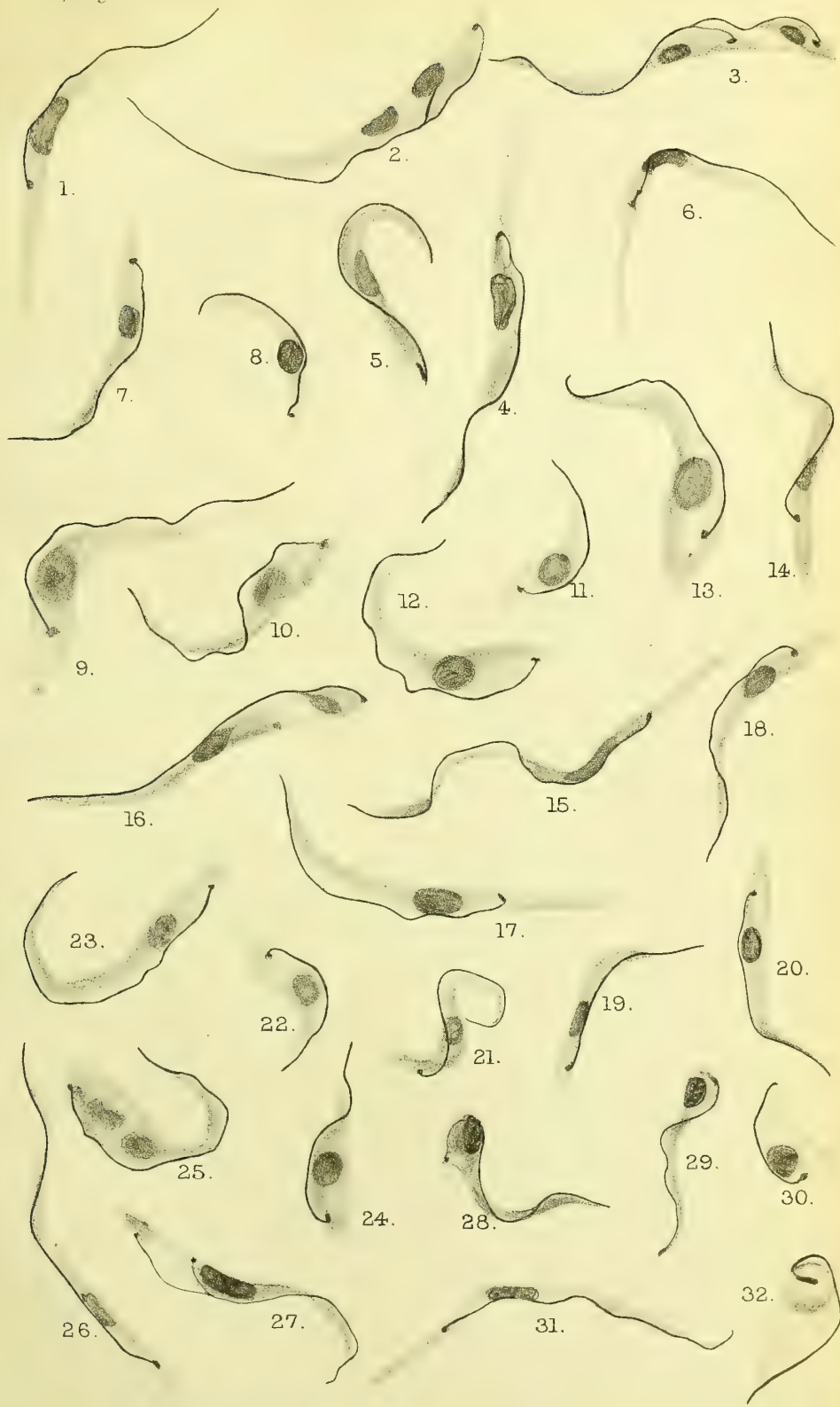


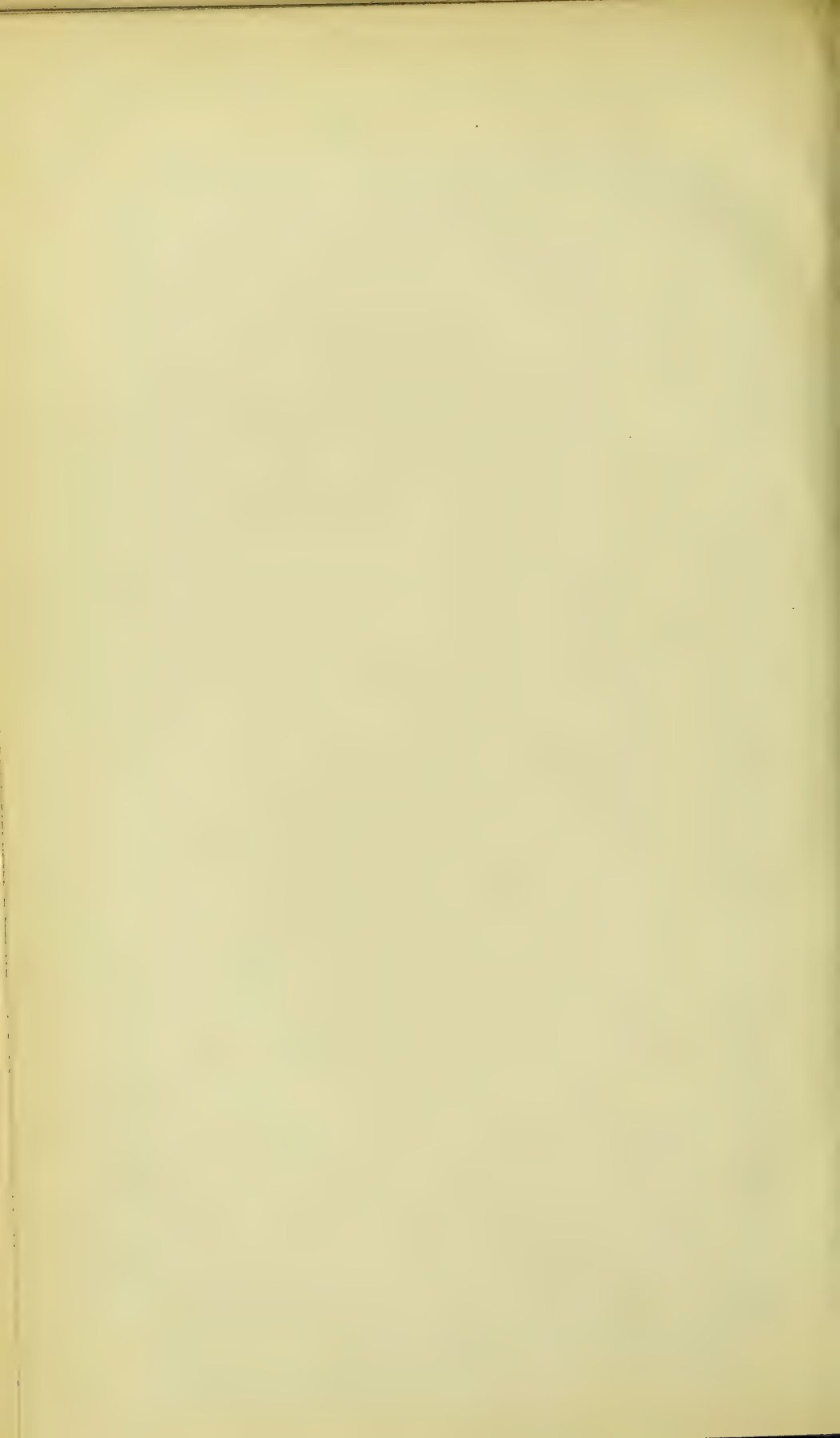


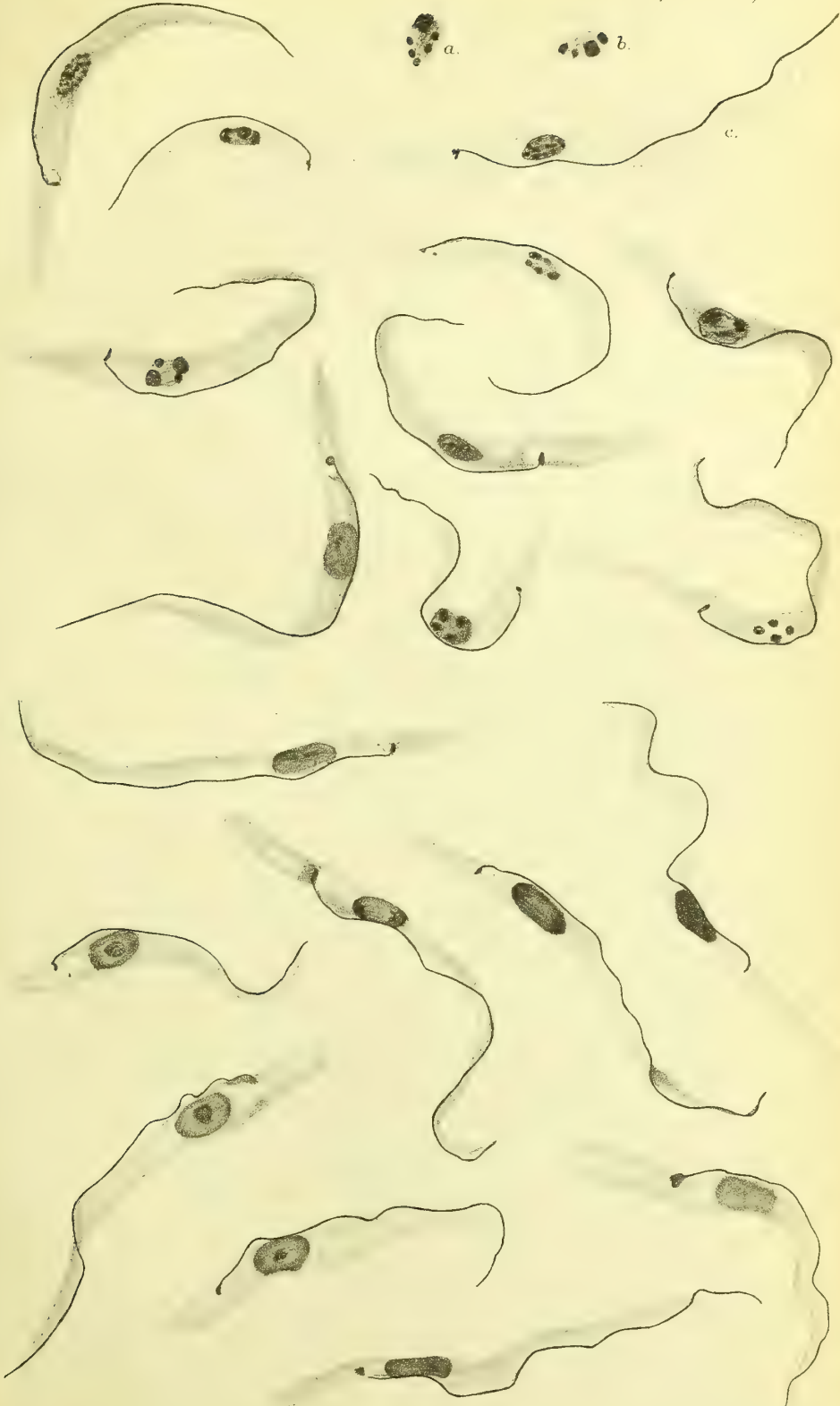


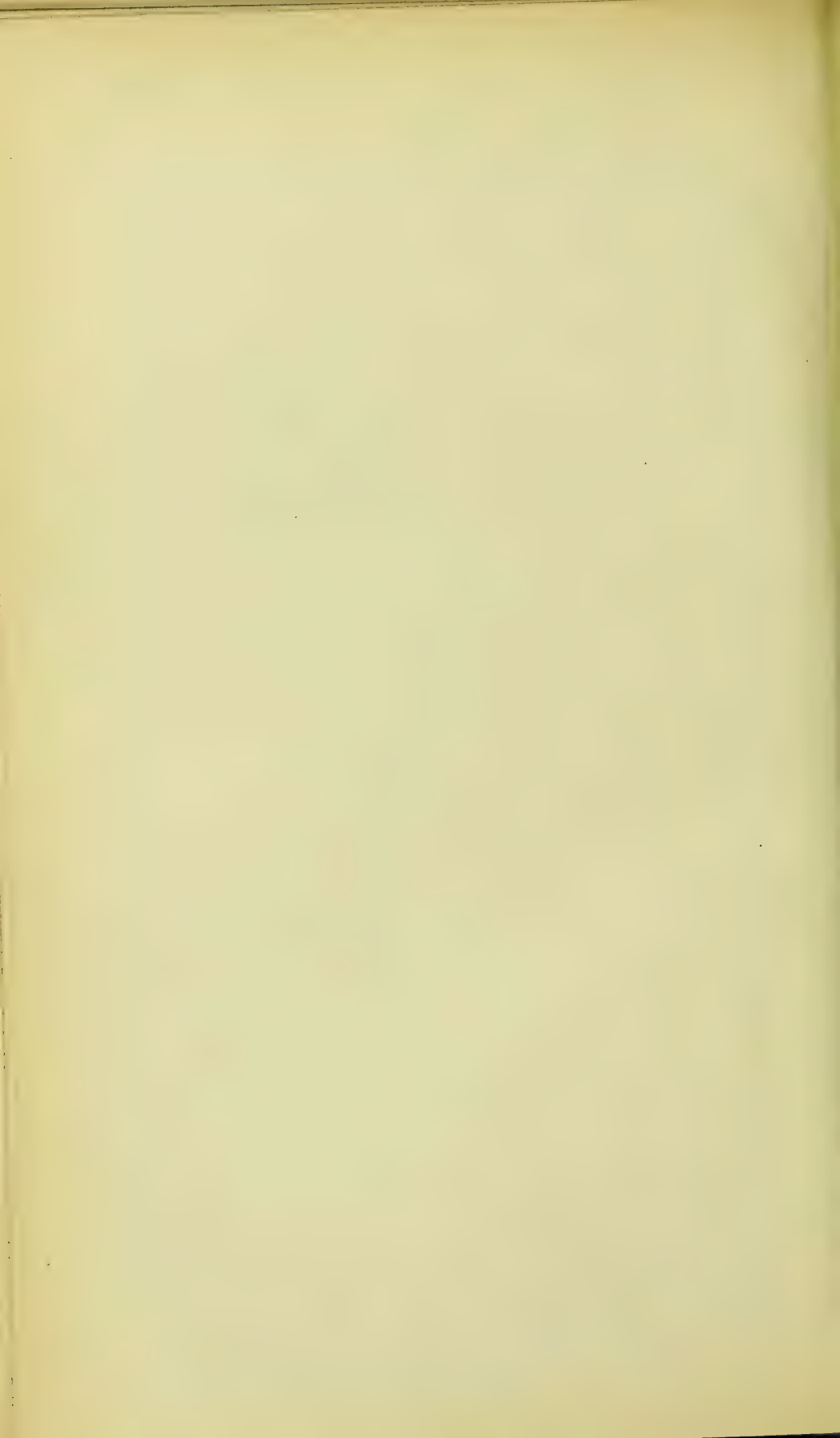


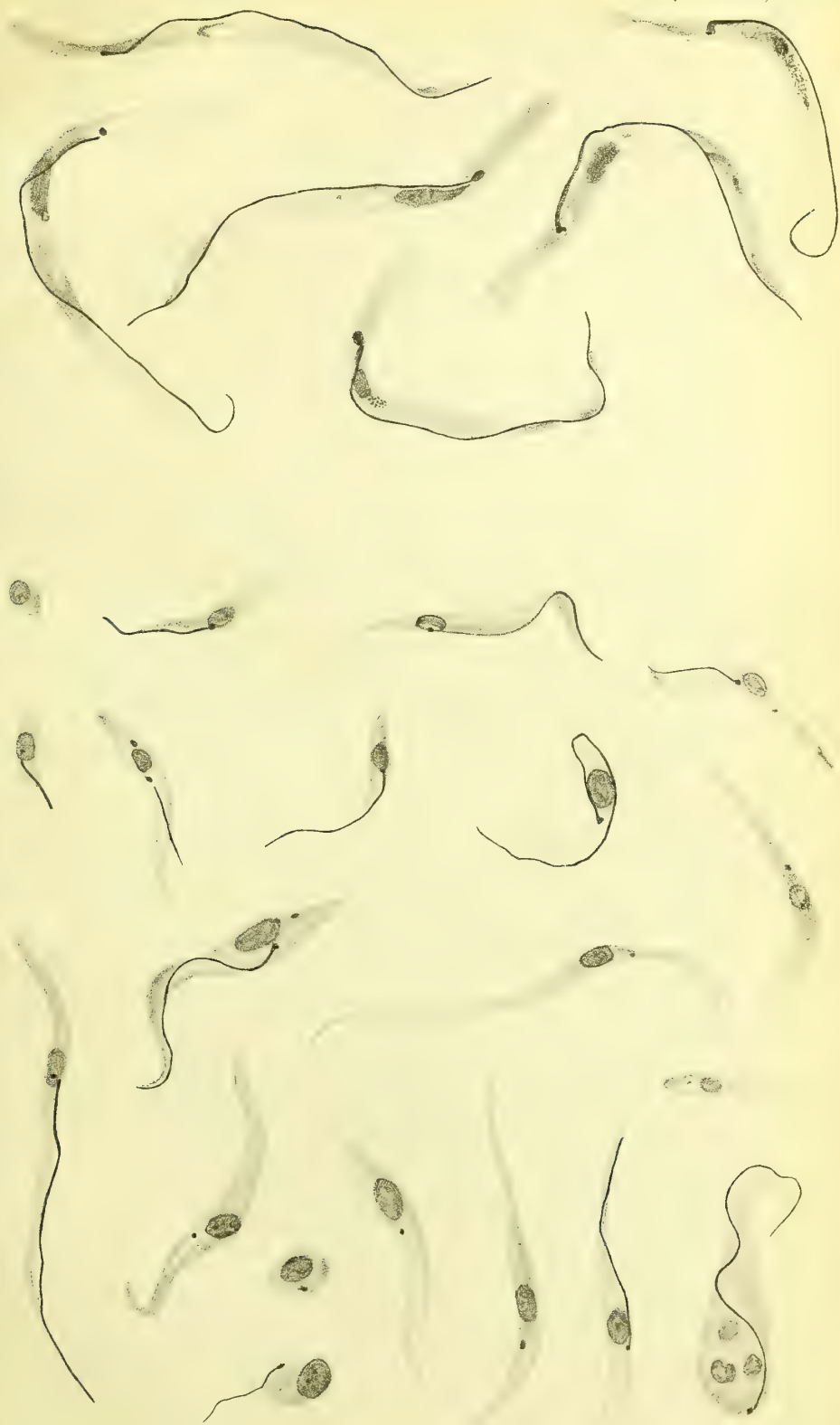


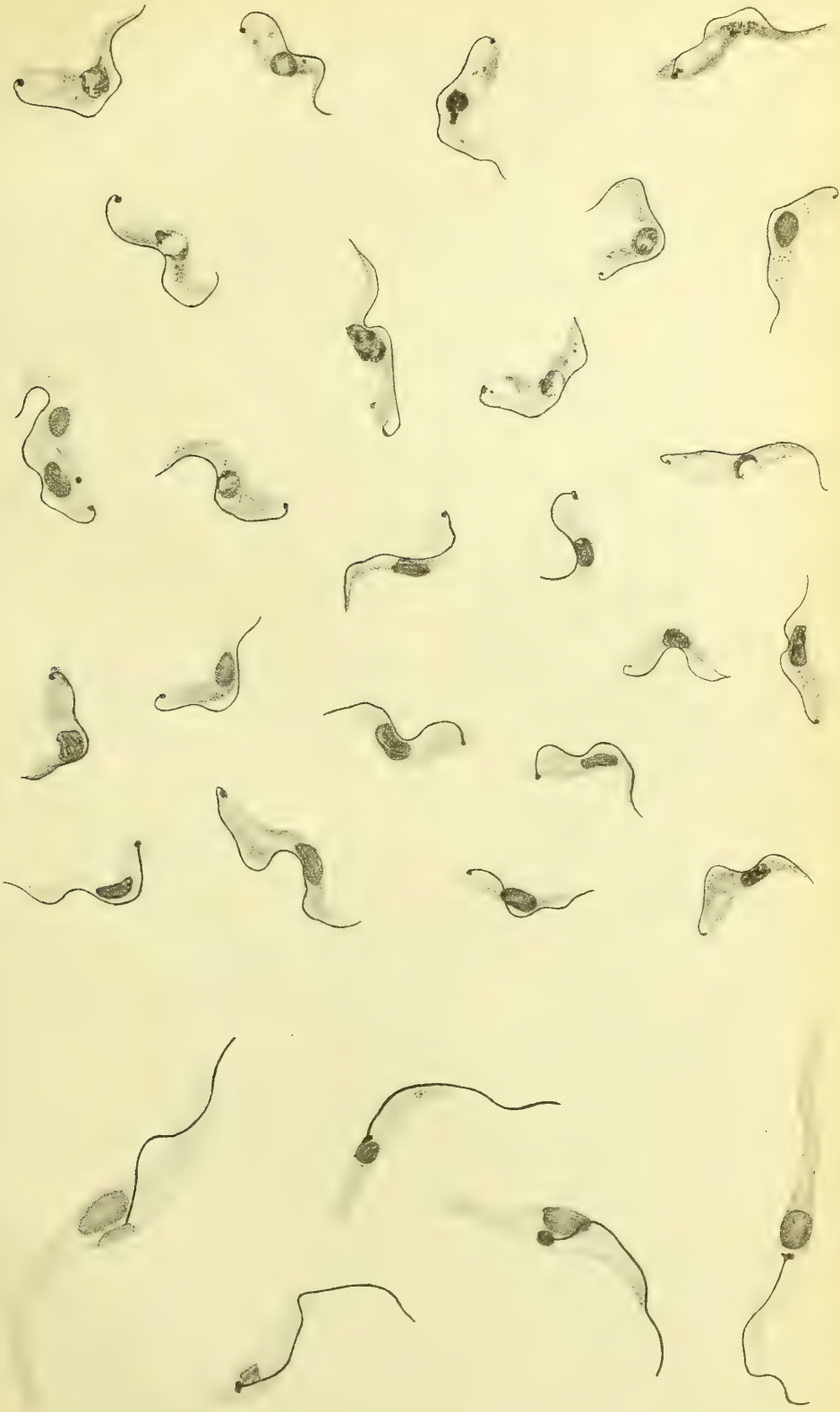
















Experiment 426.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1910. Dec. 19-20	1	53 flies fed on infected monkey, 174.			Trypanosomes present in fair numbers: first day on which trypanosomes found in monkey 174.
" 21	2	Starved 48 hours.			Flies kept in incubator.
" 22 to 1911, Feb. 18	3-61	Fed on healthy monkey, 440.		—	Feb. 20th, two remaining flies dissected.
Feb. 19-20	62-63	Flies starved.			

Remarks.—Three positive flies were found during this experiment, one on the first, and two on the 9th days. The gut-contents of these flies were not injected.

Table IX.—Fly Dissections of the above Experiments.

Experiment No.		Days of Experiment.												Totals.
		1-5	5-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	
66	Dissected	9	3	3	5	6	3	6	1	20	—	—	—	56
	Positive	1g	—	—	—	—	—	—	—	—	—	—	—	1
	Injected	—	—	—	—	—	—	—	—	—	—	—	—	—
67	Dissected	3	9	32	20	5	4	—	—	2	—	—	—	75
	Positive	2g	—	—	1g	—	1g	—	—	1g. p.v.	—	—	—	7
	Injected	—	—	—	—	—	—	—	—	—	—	—	—	—
167	Dissected	1	3	3	2	14	9	21	32	3	1	3	—	92
	Positive	1g	—	—	—	—	—	1 {g P	1 {g p.v. P	—	—	—	—	3
	Injected	—	—	—	—	—	—	—	—	—	—	—	—	—
168	Dissected	—	—	2	—	4	12	5	8	5	6	13	7	62
	Positive	—	—	—	—	—	—	—	—	—	—	1 {g p.v. P	—	1
	Injected	—	—	—	—	—	—	—	—	—	—	—	—	—
343	Dissected	7	1	3	1	7	12	1	2	5	1	—	6	46
	Positive	—	—	—	—	—	—	—	—	—	—	—	—	—
	Injected	—	—	—	—	—	—	—	—	—	—	—	—	—
349	Dissected	2	3	—	1	21	1	3	5	7	5	3	—	51
	Positive	—	2g	—	—	1g	—	1 {g p.v. P	—	1 {g P	—	—	—	5
	Injected	—	—	—	—	—	—	—	—	—	—	—	—	—
426	Dissected	7	13	5	6	3	7	3	1	—	—	—	—	45
	Positive	1g	{ 1g 1g. p.v.	—	—	—	—	—	—	—	—	—	—	3
	Injected	—	—	—	—	—	—	—	—	—	—	—	—	—
Totals	Dissected	29	32	48	35	60	48	39	49	42	13	19	13	427
	Positive	5	4	2	1	1	1	2	1	2	—	1	—	20

g = gut infection. p.v. = proventriculus infection. P = proboscis infection.

Site of Infection in Infected Flies.

In the above table the positive flies are marked according to the part of the fly which was found to be infected.

As already noted under the two previous species, the gut appears to be the constant site of infection. The proventriculus was found definitely infected in five of the six positive flies dissected after the 50th day of the experiment. In five of these flies the proboscis was also infected, the flagellates being attached in clusters to the labrum, while a few were seen in the hypopharynx in a single instance—the 59-day fly of Experiment 349. An infection of the proboscis was never met with in flies dissected before the 50th day of the experiment. The salivary glands were never found infected. A total infection of the alimentary tract, including proboscis, proventriculus, and gut—up to the point where it is joined by the Malpighian tubes—was the common type of infection after the 50th day.

Morphology of Flagellates in the Fly—stained.

Polymorphism is well marked, though less so than with *T. gambiense*.

- (a.) Gut:—Types 1, 4, 6, 7, 9, 13, 14, 27, Plate 11, are most common types; 15, 26, 31 Plate 11, occur less frequently. Types 28, 29, 30, 32 vary in frequency in different flies. No forms were seen with a free flagellum. The nucleus often shows well marked grouping of the chromatin into coarse lumps or finer granules, Plate 12. The micronucleus is often rod-shaped and accompanied by a vacuole.
- (b.) Proventriculus:—Considerable uniformity of type prevails. Long forms with blunt anti-flagellar extremity and nucleus and micronucleus in close proximity are of most general occurrence. Plates 12 and 13. No forms seen having free flagellum.
- (c.) Proboscis:—Plate 13 shows forms found in the proboscis. It will be noted that the micronucleus is unusually far forward, being situated at times anterior to the nucleus.

Plate 14 is a drawing of the strain used in the above experiments.

D.—THE DEVELOPMENT OF *TRYPANOSOMA UNIFORME* IN *GLOSSINA PALPALIS*.

The strain of this trypanosome used in the following experiments was recovered from the blood of a bushbuck shot near Kibanga. A separate paper deals with the identity of the species.

Table X.—Development of *Trypanosoma uniforme* in *Glossina palpalis*.

Experiment.	Number of flies			Number of days		
	on 1st day.	on 30th day.	on 50th day.	fed on	Before flies be- came infective.	experiment lasted.
383	37	6	4	3	—	80
388	46	6	5	3	—	79
514	57	2	—	4	37	45
524	44	24	—	5	27	36
560	123	88	—	5	31	41
566	130	99	—	16	32	47

Of these six experiments four were successful.

Experiment 383.

Date.	Days of experi- ment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1910. Dec. 7-9	1-2	37 flies fed on in- fected goat, 352.			Trypanosomes present in goat 352.
" 10-11	3-4	Flies starved.			
" 12-	5-79	Fed on healthy goat, 397.		—	Feb. 25, remaining fly dissected.
1911, Feb. 24					
Feb. 25	80	Starved.			

In experiment 388 the procedure was exactly similar to the above.

Remarks.—It is to be noted that the flies of Experiments 383, 388, and 514 were all fed on the same healthy goat—397. As no infected flies were found in Experiments 383 and 388, it may reasonably be assumed that the flies of Experiment 514 were the cause of the goat becoming infected. Experiments 383 and 388 are, therefore, recorded as negative.

Experiment 514.

Date.	Day of Experi- ment.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1911. Jan. 11-14	1 3	57 flies fed on in- fected bush-buck, 620.			Trypanosomes numerous in buck's blood.
" 15	4	Starved 48 hours.			
" 16-	5-44	Fed on healthy goat, 397.	+		Feb. 25. Remain- ing fly dissected.
Feb. 24.					
Feb. 25	45	Starved.			

Remarks.—The flies became infective about the 37th day; 34 flies were dissected during the experiment; 10 were found to have the proboscis infected with flagellates.

Experiment 524.

Date.	Day of Experiment.	Procedure.	Result.		Remarks
			Positive.	Negative.	
1911. Jan. 17-21	1-4	44 flies fed on bush-buck, 620.	+		Trypanosomes present in buck's blood.
" 22-23	5-6	Starved 72 hours.			
" 24 to Feb. 20.	7-34	Fed on healthy calf, 536.			Feb. 22. Remaining 23 flies dissected.
Feb. 21-22	35-36	Starved.			

Remarks.—Flies became infective about the 27th day. Of the 37 flies dissected during the experiment 13 were found to have a proboscis infection of flagellates. Probosces of six of the 10 positive flies found at the end of the experiment were pooled and injected subcutaneously into a healthy goat—624. *Trypanosoma uniforme* was found in this goat 29 days afterwards.

Experiment 560.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1911. Jan. 31- Feb. 4.	1-4	123 flies fed on calf, 481.	+		Trypanosomes present in small numbers in calf 481.
Feb. 5	5	Starved 48 hours.			
Feb. 6- Mar. 9.	6-37	Fed on healthy calf, 573.			March 11 & 13 remaining 79 flies dissected.
Mar. 10-11	38-39	Starved.			

Remarks.—Flies became infective about 31st day. 115 flies were dissected during the experiment, and of these 23 were found to have the proboscis infected. No injections were done.

Experiment 566.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1911. Feb. 3-18	1-15	130 flies fed on calf 481.	+		On March 21 & 22 remaining 82 flies dissected.
" 19-20	16-17	Starved 72 hours.			
" 21- Mar. 14.	18-39	Fed on healthy calf 616.			
" 15	40	Starved 24 hours.			
" 16-18	41-43	Fed on normal monkey 691.			
" 19-22	44-47	Starved.			

Remarks.—Flies became infective about the 32nd day. 107 flies were dissected during the experiment and of these 10 were

found to have the proboscis infected. Monkey 691 died before any conclusions could be drawn from this part of the experiment. Positive flies were not injected.

Table XI.—Fly Dissections of the above Experiments.

Experiment No.	—	Days of Experiment.										Total.
		1-5	5-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	
383	Dissected	5	5	10	5	—	1	—	2	2	—	30
	Positive	—	—	—	—	—	—	—	—	—	—	—
	Injection	—	—	—	—	—	—	—	—	—	—	—
388	Dissected	3	1	31	2	—	2	1	—	3	—	43
	Positive	—	—	—	—	—	—	—	—	—	—	—
	Injection	—	—	—	—	—	—	—	—	—	—	—
514	Dissected	—	3	21	8	1	1	—	—	—	—	34
	Positive	—	1	5	3	—	1	—	—	—	—	10
	Injection	—	—	—	—	—	—	—	—	—	—	—
524	Dissected	—	3	3	7	24	—	—	—	—	—	37
	Positive	—	—	2	1	10	—	—	—	—	—	13
	Injection	—	—	—	—	—	—	—	—	—	—	—
560	Dissected	1	2	10	15	50	37	—	—	—	—	115
	Positive	—	—	—	1	16	6	—	—	—	—	23
	Injection	—	—	—	—	—	—	—	—	—	—	—
566	Dissected	1	3	1	3	13	86	—	—	—	—	107
	Positive	—	—	—	—	1	9	—	—	—	—	10
	Injection	—	—	—	—	—	—	—	—	—	—	—
Totals	Dissected	10	17	76	40	88	127	1	2	5	—	366
	Showing Flagellates.	—	1	7	5	27	16	—	—	—	—	56

When this table is compared with similar ones dealing with the other species of trypanosomes, it will be noted that the number of infected flies is much higher in the present instance. It will also be seen that, with *T. uniforme*, flies showing infection are not so frequently met with during the early days of the experiment.

Site of Infection in Infected Flies.

In this trypanosome the infection has always been limited to the proboscis. Flagellates either free in the lumen of the proboscis or attached in clusters to the labrum are to be found. The hypopharynx in flies found positive during the later stages of the experiments usually contained flagellates. Here the forms were smaller and more uniform in size as compared with the types met with in the main tube of the proboscis.

Morphology of Flagellates in the Fly—stained.

Plates 14 and 15 are drawings of flagellates from the probosces of infected flies. The marked feature here is the position of the micronucleus which always is situated close to the nucleus. The micronucleus in some of the drawings is seen to be large.

Conclusions:—*Trypanosoma gambiense*, *brucei*, *pecorum*, and *uniforme* undergo a development in laboratory-bred *Glossina palpalis*.

2. In the case of *Trypanosoma gambiense* and *brucei* the gut is the chief site of development.

3. In *Trypanosoma pecorum* the proboscis in addition to the gut is usually infected after the 50th day from the infected feed.

4. Development of *Trypanosoma uniforme* in the fly is limited to the proboscis.

DESCRIPTION OF PLATES.

All drawings were made with the aid of the Camera lucida and at a magnification of 2,000 diameters. All preparations were fixed with osmic acid and absolute alcohol and stained by Giemsa.

Plate 4.—Types of *Trypanosoma gambiense* in the gut of *G. palpalis* from 13th–22nd days after the first infective feed.

Plate 5.—*T. gambiense* as seen in the gut of a 41-day fly of positive experiment 190. The proventriculus of this fly was negative.

Plate 6.—*T. gambiense* in the proventriculus of a 31-day fly of positive experiment 469. *T. gambiense* in the proventriculus of a 45-day fly of positive experiment 88.

Plate 7.—A flagellate found in a 38-day fly of positive experiment 469 (see text).

Plate 8.—*T. brucei* in the gut of a 45-day fly of experiment 42.

Plate 9.—Types of *T. brucei* in gut of *G. palpalis*, and in blood of monkey 367.

Plate 10.—*T. brucei* in blood of monkey 367, and in blood of guinea-pig 380.

Plate 11.—*T. pecorum* in gut of a 61-day fly in negative experiment 167.

Plate 12.—*T. pecorum* in gut of 9-day fly in experiment 426. (*a* and *b* specimen nuclei. *c* prevailing type in gut of 9-day fly.) *T. pecorum* in proventriculus of a 96-day fly of positive experiment 168.

Plate 13.—*T. pecorum* in proventriculus of fly of plate 11, and in proboscis of fly of plate 11.

Plate 14.—*T. pecorum* in blood of monkey 174, and *T. uniforme*.

Plate 15.—*T. uniforme* in proboscis of 38-day fly in positive experiment 567. *a-d* foreign bodies, apparently of nature of spores, occasionally found on slides. *T. uniforme* in proboscis of 36-day fly in positive experiment 524.

8.—AN ANTELOPE TRYPANOSOME.

By Capt. A. D. FRASER, R.A.M.C., and Dr. H. L. DUKE.

(Plates 16-25.)

Introduction.

When examining animals which frequent the Lake Shore with the view of ascertaining whether they are to be found naturally infected with trypanosomiasis, the species of trypanosome which forms the subject of this paper was discovered. It was first seen in blood smears made from a bush-buck shot near Kibanga.

A short time afterwards two bush-buck were shot in the same neighbourhood, and their blood was injected into a healthy goat. Ten days after the injection trypanosomes appeared in the goat's blood. This strain was the one which was studied.

A situtunga shot at Kibanga was also found to be infected.

In blood smears of a bush-buck and of a reed-buck sent from Toro by Dr. van Someren and marked 'morsitans area' a closely similar, if not identical, trypanosome was found.

Morphology. A. Living, Unstained.

When observed in fresh blood, the trypanosome is seen to be actively motile. It moves about the field usually with the flagellar end in front, and it is not uncommon for it to travel across the field of the microscope when using a low magnification. The rate at which it goes is never, however, so rapid as to make it difficult to follow. As the trypanosome works its way among the blood corpuscles the flagellum and the body show a marked quivering motion. These translatory movements are interrupted by periods when the trypanosome remains near the same spot in the field. A lashing to and fro motion of the flagellum and anterior part of the body is then usual. The red blood corpuscles are pushed aside, and the trypanosome is left in a small clear space.

B. Fixed and Stained.

A small characteristic trypanosome. Typically, the anti-flagellar end is blunt and somewhat rounded, while the greatest transverse diameter is approximately midway between the two nuclei, giving a swollen appearance to this end of the trypanosome. From the nucleus the body tapers somewhat abruptly to the flagellar extremity.

In length the trypanosome varies from 11·5-20·5 microns, the average being 15·63 microns. The greatest breadth of the posterior third may reach 5 microns; the average breadth across the nucleus is 2·3 microns.

The following table gives the average length of this trypanosome in the ox, goat and bushbuck. Twenty trypanosomes are drawn and measured from each preparation.

Table I.—Measurements of the Antelope Trypanosome.

No. of experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
620	Bushbuck	Osmic acid	Giemsa	14·15	16·50	11·50
481	Ox	"	"	15·85	17·50	13·25
3,311 } 536 } 23,211 }	"	"	"	15·47	17·25	12·50
536 }	"	"	"	16·96	19·50	13·50
573	"	"	"	15·86	17·50	14·50
352	Goat	"	"	14·36	17·75	12·50
397	"	"	"	15·70	17·50	13·50
431	"	"	"	15·90	18·00	14·25
504	"	"	"	14·77	17·00	12·50
616	Ox	"	"	17·32	20·50	15·00
				15·63	20·5	11·5

The following table shows the distribution in respect to length of 200 individuals of this species of trypanosome:—

Table II.—Distribution in respect to Length of 200 individuals.

No. of experiment.	Microns.										Average in microns.
	11	12	13	14	15	16	17	18	19	20	
620	1	—	6	8	4	1	—	—	—	—	14·15
481	—	—	2	4	2	7	5	—	—	—	15·85
3.3.11 } 536 }	—	1	—	4	5	8	2	—	—	—	15·47
23.2.11 } 536 }	—	—	1	1	3	4	5	2	4	—	16·96
573	—	—	—	4	6	6	4	—	—	—	15·86
352	—	4	5	3	3	4	—	1	—	—	14·36
397	—	—	1	3	5	9	2	—	—	—	15·70
431	—	—	—	3	8	5	1	3	—	—	15·90
504	—	1	1	9	6	1	2	—	—	—	14·77
616	—	—	—	—	2	5	4	7	1	1	17·32
Totals ...	1	6	16	39	44	50	25	13	5	1	—
Percentage ...	0·5	3	8	19·5	22	25	12·5	6·5	2·5	0·5	—

Nucleus.—Is round or oval and situated about the middle of the body. Usually it extends from the root of the undulating membrane to the opposite edge of the body. In structure it is not homogeneous, chromatin masses of various sizes being arranged around the periphery.

Micronucleus.—A large and often rounded structure, typically pushed up against the extreme anti-flagellar end of the trypanosome. Occasionally a clear point may be seen at its centre.

Undulating membrane.—Only moderately developed. In the posterior $\frac{1}{3}$ rd of the trypanosome its flagellar edge has an intracorporeal course of varying length, appearing on the edge of the body somewhere between the two nuclei. This flagellar portion is usually, though not always, prolonged anteriorly into a more or less marked free flagellum, which may attain a length of 5 microns.

Protoplast.—Is, as a rule, homogeneous, though in certain individual host-animals coarse granules appear in varying numbers. These granules stain deeply like chromatin, and are placed irregularly on either side of the nucleus.

A vacuole may be present near the micronucleus and others are at times scattered throughout the protoplast.

Animals susceptible to the Antelope Trypanosome.

Table III. shows that cattle, bushbuck, goats and sheep are susceptible. It is too early to say what effect the trypanosome will have upon these animals. Up to the present, however, none of them appear to be the worse of the infection. There was no

evidence that Goat, Experiment 504, died of trypanosomiasis. Monkeys, pigs, dogs, cats, guinea-pigs and white rats all proved to be refractory.

Table III.—Animals susceptible to the Antelope Trypanosome.

Date.	No. of experiment.	Source of virus.	Period of incubation in days.	Duration of disease in days.*	Remarks.
CATTLE.					
1911.					
Jan. 3	481	Goat 352 ...	11	—	Still alive after 87 days.
" 10	501	Bushbuck 620	17	—	" " 80 "
Feb. 20	536	Fly infection	?	—	" " 39 "
Mar. 10	573	" "	?	—	" " 21 "
" 15	616	" "	?	—	" " 16 "
BUSHBUCK.					
Jan. 3	620	Goat 352 ...	7	—	Still alive after 87 days.
GOAT.					
1910.					
Nov. 24	352	Kibanga Bushbuck.	10	—	Still alive after 125 days.
Dec. 12	431	Goat 352 ...	—	—	1st injection ; negative for 64 days.
1911.					
Feb. 23	431	Ox 536 ...	8	—	2nd injection ; still alive after 36 days.
Jan. 10	504	Bushbuck 620	30	71	Cause of death unknown.
" 24	397	Fly infection	?	—	Still alive after 35 days.
" 24	624	Fly infection	29	—	" " 37 "
SHEEP.					
Jan. 23	534	Calf 481 ...	8	—	Still alive after 67 days.
BUSH-PIG.					
Jan. 10	502	Bushbuck 620	—	—	1st injection ; negative for 31 days.
Feb. 23	502	Ox 536 ...	—	—	2nd injection ; negative for 29 days.
" 23	625	" ...	—	—	Negative for 29 days.
DOG.					
Jan. 23	535	Calf 481 ...	—	—	1st injection ; negative for 31 days.
Feb. 23	535	Ox 536 ...	—	—	2nd injection ; negative for 32 days.
" 23	626	" ...	—	—	Negative for 32 days.
CAT.					
Dec. 21	436	Goat 352 ...	—	—	Negative for 32 days.

* Duration of disease includes incubation period where this is known.

Date.	No. of experiment.	Source of virus.	Period of incubation in days.	Duration of disease in days.*	Remarks.
MONKEYS.					
1910.					
Dec. 7	377	Goat 352 ...	—	—	Negative for 69 days.
" 21	432	" ...	—	—	" " 44 "
1911.					
Jan. 10	503	Bushbuck 620	—	—	" " 49 "
Feb. 23	627	Ox 536 ...	—	—	" " 16 "
					Monkey died.
" 23	628	" ...	—	—	Negative for 32 days.
GUINEA-PIG.					
1910.					
Dec. 7	378	Goat 352 ...	—	—	Negative for 69 days.
" 21	433	" ...	—	—	" " 65 "
1911.					
Jan. 10	505	Bushbuck 620	—	—	" " 49 "
" 10	506	" "	—	—	" " 49 "
WHITE RATS.					
1910.					
Dec. 7	379	Goat 352 ...	—	—	Negative for 48 days.
" 21	434	" ...	—	—	" " 55 "
1911.					
Jan. 10	507	Bushbuck 620	—	—	" " 45 "
" 10	508	" "	—	—	" " 45 "
EDIBLE RAT.					
1910.					
Dec. 21	435	Goat 352 ...	—	—	Negative for 55 days.

* Duration of disease includes incubation period where this is known.

These animal reactions indicated that the trypanosome was allied to *Trypanosoma vivax*, *cazalboui* and *nanum*.

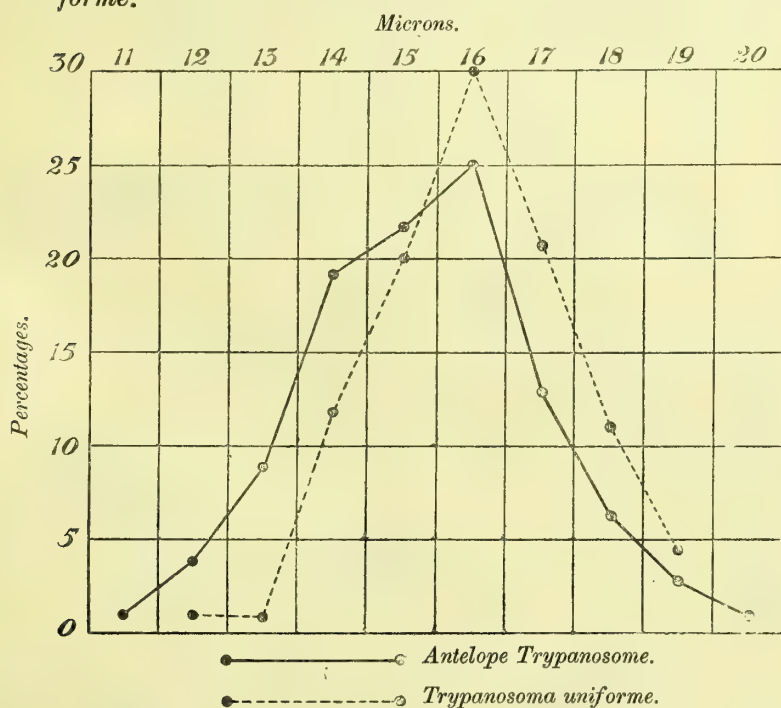
Colonel Sir David Bruce, C.B., F.R.S., kindly sent us the papers on *Trypanosoma vivax* by the Sleeping Sickness Commission of the Royal Society, 1908-10. This enabled us to satisfy ourselves that the trypanosome we were dealing with differed from that species, particularly in its movements and measurements. A few weeks ago, he presented us with papers on *T. nanum* and a new trypanosome—*T. uniforme*. With the evidence available from this latter paper, it was at once obvious that the antelope trypanosome was very similar, if not identical, to *T. uniforme*.

The morphology in the living condition appeared to be the same in both trypanosomes.

The measurements given for *T. uniforme* are:—Average length 16 microns, maximum 19 microns and minimum 12 microns. The antelope trypanosome measurements given above are:—Average length 15·63 microns, maximum 20·5 microns and minimum 11·5 microns. The measurements of the two trypanosomes therefore correspond.

The distribution in respect to length of 200 individuals of *T. uniforme* is compared with the same number of the antelope trypanosome in the following chart.

Chart I.—Curves representing Distribution, by Percentages, in respect to length of the Antelope Trypanosome and *T. uniforme*.



As regards morphology, there can be little doubt as to the identity of this trypanosome and *T. uniforme*.

Cultivation of the Antelope Trypanosome.

Two tubes of blood agar were inoculated with this trypanosome. They remained sterile for six weeks and were then discarded.

The Carrier of the Antelope Trypanosome.

In a previous paper it has been shown experimentally that this species of trypanosome could be transmitted fairly readily from infected to healthy animals by *Glossina palpalis* which had been bred in the laboratory. Of six experiments which were carried out, four were successful. The number of days which elapsed after the flies were first fed on an infected animal before they became infective varied from 27 to 37 days. The infection in the fly was always found to be limited to the proboscis.

It having, then, been shown that the antelope frequenting the Lake shore were infected and that laboratory-bred *Glossina palpalis* were capable of acting as a carrier, it seemed probable that the *Glossina palpalis* caught on the Lake shore in the same neighbourhood as the buck had been got from might be naturally infected. In order to put this to the test, flies were brought to Mpumu and there fed on a healthy goat.

The details of the experiment are as follows:—

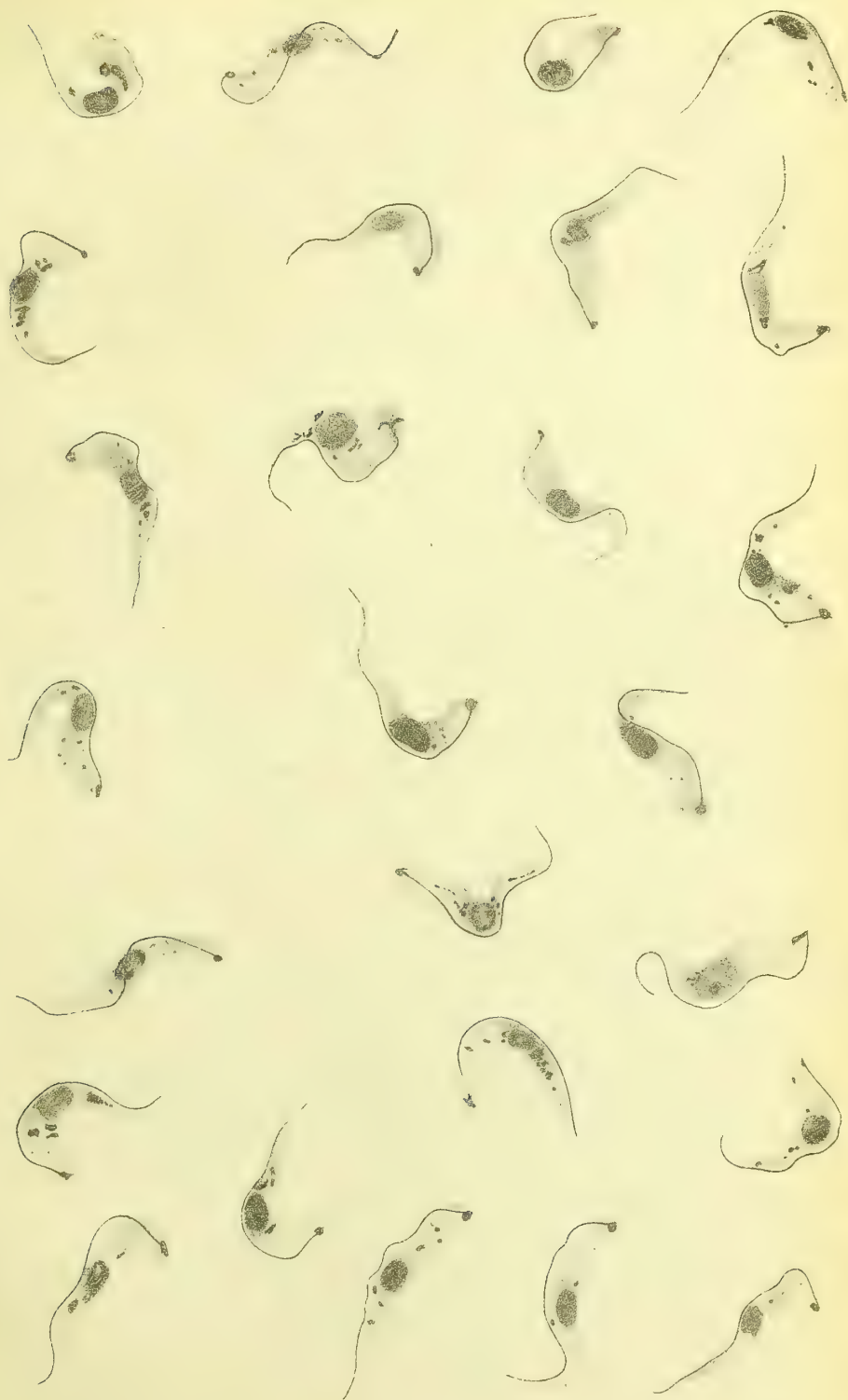
Experiment 663. Goat.

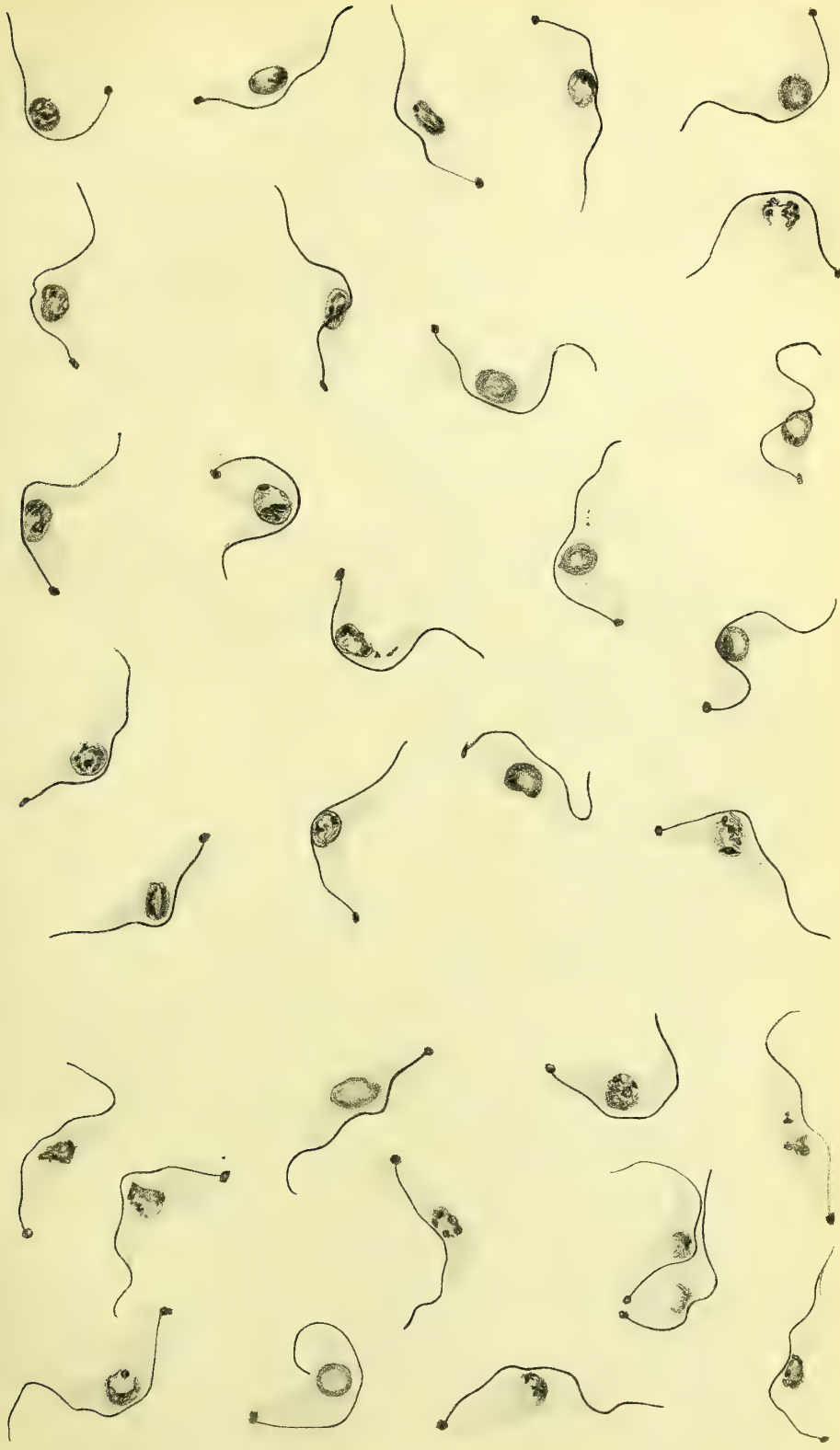
To ascertain whether *Glossina palpalis* caught near Kibanga are naturally infected with the antelope trypanosome.

Date.				Number of flies fed on.	Result.	
					<i>Antelope T.</i>	<i>T. vivax.</i>
1911.						
March	7	...	...	200	—	—
"	9	...	...	200		
"	10	...	...	150	—	—
"	11	...	...	250		
"	13	...	...	50	—	—
"	14	...	...	—	—	—
"	15	...	...	—	—	—
"	16	...	...	170	—	—
"	17	...	...	—	—	—
"	18	...	...	—	—	—
"	19	...	...	—	—	—
"	20	...	...	—	—	—
"	21	...	...	100	—	—
"	22	...	...	200	—	—
"	23	...	...	—	+	—
"	24	...	...	—	+	—
"	25	...	...	—	++	—
"	27	...	...	—	—	—
"	28	...	...	—	—	—
"	29	...	...	—	—	+
"	30	...	...	—	—	—
"	31	...	...	—	—	+
April	1	...	...	—	+	+
"	2	...	...	—	+	++
"	3	...	...	—	+	++

Remarks.—On March 23rd, the antelope trypanosome appeared in the goat's blood (*see* Plate 23). Allowing 7 days for incubation in the goat, the infection would appear to have taken place about March 16th, and must therefore have been caused by 1,020 flies. On March 29th a much more active trypanosome was seen in the goat's blood. This proved to be *Trypanosoma vivax*. The infection with this trypanosome probably occurred on March 22nd—being caused by 1,320 flies. Plate 24 shows *T. vivax* and the antelope trypanosome in the same preparation of the goat's blood taken on April 1st. On the same date it was























easy to distinguish the two species of trypanosomes in the living state by their characteristic movements.

The above experiment thus showed that Lake-shore *Glossina palpalis* are naturally infected with the antelope trypanosome, which is believed to be *T. uniforme*. It also showed that these flies still retain their infectivity with *Trypanosoma vivax*. Further, it is additional evidence that *Trypanosoma uniforme* and *vivax* are different species.

Conclusions.

1. This trypanosome, which is of fairly frequent occurrence among Lake-shore antelope, is *Trypanosoma uniforme*.

2. The available evidence points to *Glossina palpalis* as being the carrier of this species of trypanosome.

3. *Glossina palpalis* caught on the Lake shore are naturally infected with *Trypanosoma uniforme*.

DESCRIPTION OF PLATES.

Plates 16-25. *T. uniforme* in the blood. Fixed osmic acid and absolute alcohol and stained with Giemsa. All drawings made at magnification of 2,000 diameters.

Plate 16	...	<i>T. uniforme</i> in blood of Bushbuck	620.
Plate 17	...	<i>T. uniforme</i> " " Ox	481.
Plate 18	...	<i>T. uniforme</i> " " "	536.
Plate 19	...	<i>T. uniforme</i> " " "	536 and 573.
Plate 20	...	<i>T. uniforme</i> " " Goat	352.
Plate 21	...	<i>T. uniforme</i> " " "	397 and 431.
Plate 22	...	<i>T. uniforme</i> " " "	431 and 504.
Plate 23	...	<i>T. uniforme</i> " " Ox	616 and Goat 663.
Plate 24	...	<i>T. uniforme</i> (a and b) and <i>T. vivax</i> in blood of Goat	663.
Plate 25	...	<i>T. uniforme</i> in blood of wild Lake-shore Situtunga.	

9.—DURATION OF THE INFECTIVITY OF THE GLOSSINA PALPALIS AFTER THE REMOVAL OF THE LAKE-SHORE POPULATION.

By Capt. A. D. FRASER, R.A.M.C., and Dr. H. L. DUKE.

In a paper on this subject the Sleeping Sickness Commission of the Royal Society, 1908-09, reported a series of experiments which showed that *Glossina palpalis* caught on the uninhabited Lake shore remained capable of infecting animals with *Trypanosoma gambiense* for two years after the inhabitants had been removed from within two miles of the Lake shore.

The experiments described in this paper were carried out to ascertain whether the flies still retained their infectivity.

The flies employed in the experiments and noted in the details as 'Kibanga Flies,' were, as a rule, caught near the point to the west of the entrance to Buka Bay. It was found difficult to obtain flies in sufficient numbers from the fly area near the entrance of the Bay on the east. The flies in this area, which is fairly limited in extent, appear to have considerably diminished, —most probably due to the collection of large numbers of flies and their pupæ during the past two years.

Other localities, where observations were made, were Nsanga and Bukafu, both of which are old landing places on the mainland situated respectively about 10 and 20 miles to the west and east of Kibanga. It will be seen in the details of the experiments which of them were carried out at these places.

It was considered desirable to test the flies on the Islands. Damba had already been examined by Hamerton and Bateman. A small island was thought to serve the purpose of the experiments best, as the chances of there being still a few inhabitants remaining on an island from whom flies might derive the infection seemed to be greater in the larger islands. Nsadzi and Kimmi were chosen as being most suitable.

The number of flies given in the details of the experiments are the actual numbers of active flies in the cages when they were put on the monkeys. In at least 90 per cent. of the flies it was apparent from the condition of the abdomen that they had fed. In another paper an experiment is described which appeared to show that the act of piercing the skin by the proboscis of an infected fly was sufficient in itself to bring about an infection. Actual feeding did not seem to be essential. It was not, therefore, deemed necessary to attempt to distinguish between the number of flies which were put on the monkeys and the number which fed upon them.

The experiments are so important that they are given in detail.

Experiment 21. Monkey.

To ascertain if *Glossina palpalis* caught on the Lake shore near Kibanga are capable of giving rise to Sleeping Sickness in healthy monkeys.

Kibanga Flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Aug. 8	44	—	Aug. 12	—	—
" 9	—		" 13	200	
" 10	305		" 15	500	
" 11	40		" 16	—	++

Remarks.—The result of this experiment was positive. One of the 44 flies fed on August 8th must have caused the infection.

Experiment 48. Monkey.

Kibanga Flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Aug. 16	260		Sept. 28	—	—
" 17	230		" 29	—	—
" 19	220	—	" 30	—	—
" 22	200		Oct. 3	—	—
" 23	160	—	" 4	—	—
" 24	40		" 5	—	—
" 25	120		" 6	—	—
" 26	120	—	" 7	—	—
" 29	170		" 8	—	—
" 30	230	—	" 11	—	—
" 31	105	—	" 12	—	—
Sept. 1	—	—	" 14	60	—
" 2	—	—	" 17	130	—
" 3	—	—	" 19	130	—
" 5	—	—	" 20	—	—
" 6	—	—	" 21	—	—
" 7	—	—	" 22	—	—
" 8	—	—	" 24	—	—
" 9	—	—	" 25	—	—
" 10	—	—	" 26	—	—
" 12	130	—	" 27	—	—
" 13	60	—	" 28	—	—
" 14	—	—	" 29	—	—
" 15	250	—	" 31	—	—
" 16	50	—	Nov. 1	—	—
" 17	—	—	" 2	—	—
" 19	—	—	" 3	—	—
" 20	220	—	" 4	—	—
" 21	175	—	" 5	—	—
" 22	—	—	" 7	—	—
" 23	160	—	" 8	—	—
" 24	—	—	" 9	—	—
" 26	—	—	" 10	—	—
" 27	—	—	" 11	—	—

Remarks.—Result negative. 3,220 flies failed to infect this monkey which died on November 11th, the cause of death being unknown. 3 c.c. of the blood taken from the heart at death was injected subcutaneously into a healthy monkey with negative result.

Experiment 122. Monkey.

Kibanga Flies.

Date.	No. of flies fed on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Sept. 1	210	—	Dec. 1	165	—
" 2	300	—	" 2	360	—
" 5	300	—	" 3	430	—
" 6	—	—	" 5	500	—
" 7	—	—	" 6	660	—
" 9	360	—	" 7	350	—
" 10	—	—	" 8	100	—
" 12	—	—	" 9	400	—
" 13	—	—	" 10	—	—
" 14	—	—	" 12	—	—
" 15	—	—	" 13	—	—
" 16	—	—	" 14	—	—
" 17	—	—	" 15	—	—
" 19	—	—	" 16	—	—
" 20	—	—	" 17	170	—
" 21	—	—	" 19	—	—
" 22	—	—	" 20	—	—
" 23	—	—	" 21	150	—
" 27	—	—	" 22	250	—
" 30	—	—	" 23	300	—
Oct. 4	—	—	" 24	—	—
" 7	—	—	" 26	—	—
" 11	—	—	" 27	200	—
" 14	—	—	" 28	60	—
" 18	—	—	" 29	—	—
" 21	—	—	" 30	200	—
" 25	—	—	" 31	100	—
" 28	—	—			
Nov. 1	—	—	1911.		
" 4	—	—	Jan. 2	—	—
" 8	—	—	" 3	—	—
" 10	120	—	" 4	—	—
" 11	—	—	" 5	—	—
" 14	480	—	" 6	—	—
" 15	—	—	" 7	—	—
" 18	—	—	" 9	—	—
" 22	—	—	" 10	—	—
" 23	—	—	" 11	—	—
" 24	—	—			
" 25	—	—			
" 26	360	—			
" 28	—	—			
" 29	—	—			
" 30	100	—			

Remarks.—Result negative. 6,625 flies were fed on this monkey without producing an infection. The monkey died on January 12th from some unknown cause. On January 6th and again on January 12th 1 c.c. of the monkey's blood was injected into white rats with negative results.

Experiment 198. Monkey.

To ascertain if *Glossina palpalis* caught on the Lake shore at Nsanga are capable of giving rise to Sleeping Sickness in healthy monkeys.

Nsanga Flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Sept. 25	95		Oct. 17	—	—
" 27	595		" 18	—	—
" 28	650		" 19	—	—
" 29	400		" 20	—	—
" 30	250		" 25	—	—
Oct. 1	400		" 28	—	—
" 4	—	—	Nov. 8	—	—
" 5	—	—	" 9	—	—
" 6	—	—	" 10	—	—
" 7	—	—	" 11	—	—
" 8	—	—	" 12	—	—
" 10	—	—	" 14	—	—
" 11	—	—	" 15	—	—
" 12	—	—	" 16	—	—
" 13	—	—	" 17	—	—
" 14	—	—	" 18	—	—
" 15	—	—			

Remarks.—Result negative. 2,390 flies failed to infect the monkey.

Experiment 199. Monkey.

Nsanga Flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Sept. 28	600		Oct. 18	—	—
" 29	300		" 19	—	—
" 30	510		" 20	—	—
Oct. 1	530		" 25	—	—
" 4	—	—	" 28	—	—
" 5	—	—	Nov. 8	—	—
" 6	—	—	" 9	—	—
" 7	—	—	" 10	—	—
" 8	—	—	" 11	—	—
" 10	—	—	" 12	—	—
" 11	—	—	" 14	—	—
" 12	—	—	" 15	—	—
" 13	—	—	" 16	—	—
" 14	—	—	" 17	—	—
" 15	—	—	" 18	—	—
" 17	—	—			

Remarks.—Result negative. 1,940 flies failed to cause infection.

Experiment 208. Monkey.

Nsanga Flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Oct. 2	350		Oct. 22	—	—
" 3	480		" 24	—	—
" 4	500		" 25	—	—
" 5	460		" 26	—	—
" 6	300		" 27	—	—
" 10	—	—	" 28	—	—
" 11	—	—	" 29	—	—
" 12	—	—	" 31	—	—
" 13	—	—	Nov. 1	—	—
" 14	—	—	" 2	—	—
" 15	—	—	" 3	—	—
" 17	—	—	" 4	—	—
" 18	—	—	" 8	—	—
" 19	—	—	" 11	—	—
" 20	—	—	" 15	—	—
" 21	—	—			

Remarks.—Result negative. 2,090 flies failed to infect monkey.

Experiment 209. Monkey.

Nsanga Flies

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Oct. 2	350		Oct. 6	300	
" 3	480		Nov. 10	—	—
" 4	450		" 11	—	—
" 5	500		" 12	—	++

Remarks.—Result positive. Infection probably occurred on October 5 and was caused by 1,780 flies.

Experiment 223. Monkey.

Nsanga flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Oct. 7	210		Oct. 13	226	
" 8	300		" 14	—	—
" 9	330		" 15	—	—
" 10	470		" 17	—	—
" 11	300		" 18	—	—
" 12	550		" 19	—	++

Remarks.—Result positive. Infection probably on October 12, 2,160 flies.

Experiment 224. Monkey.

Nsanga flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Oct. 7	390		Oct. 18	—	—
" 8	300		" 19	—	—
" 9	330		" 20	—	—
" 10	500		" 21	—	—
" 11	300		" 22	—	—
" 12	540		" 24	—	—
" 13	220		" 25	—	—
" 14	—		" 26	—	—
" 15	—	—			Oct. 26, Monkey died.
" 17	—	—			

Remarks.—Result negative. 2,580 flies failed to cause infection.

Experiments 289 and 290.

To ascertain if *Glossina palpalis* caught on the Lake shore at Bukafu are capable of giving rise to Sleeping Sickness in healthy monkeys.

In each experiment 1,750 flies were put upon monkeys between October 22 and November 7, 1910. Both monkeys were alive and apparently healthy on December 23rd and 31st respectively, trypanosomes never having been seen in their blood although examined almost daily.

Experiment 321. Monkey.

Kibanga flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1910.			1910.		
Nov. 16	230		Nov. 23	—	—
" 17	470		" 24	—	—
" 18	150	—	" 25	—	—
" 21	110		" 26	—	+
" 22	—	—			

Remarks.—Result positive. Infection probably occurred on November 18th with 850 flies.

Experiment 478. Monkey.

Kibanga flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Jan. 3	170	—	Jan. 17	415	—
" 4	130	—	" 18	375	—
" 5	84	—	" 19	200	—
" 9	110	—	" 20	300	—
" 10	300	—	Feb. 13	425	—
" 11	250	—	" 14	550	—
" 12	500	—	" 15	950	—
" 13	90	—	" 16	550	—
" 16	480	—	" 22	400	—

Remarks.—Result negative. 6,279 flies failed to infect the monkey whose blood was examined daily from January 10th to March 21st. The monkey was healthy on April 7th, 1911. On March 9th 2 c.c. of its blood was injected subcutaneously into White Rat 664 without causing an infection.

Experiment 597. Monkey.

Kibanga flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Feb. 13	425	—	Feb. 22	400	—
" 14	550	—	" 23	420	—
" 15	950	—	" 24	250	—
" 16	550	—	" 25	—	—
" 17	120	—	" 27	—	++
" 20	480	—	" 28	—	+++
" 21	400	—			

Remarks.—Result positive. Infection probably occurred on February 20th, being caused by 3,075 flies.

Experiment 633. Monkey.

Kibanga flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Feb. 23	420	—	Mar. 9	350	—
" 24	250	—	" 10	475	—
" 27	480	—	" 13	270	—
" 28	350	—	" 14	650	—
Mar. 1	400	—	" 15	400	—
" 2	500	—	" 16	400	—
" 3	300	—	" 17	}	—
" 6	375	—	Apr. 22		
" 8	475	—			

Remarks.—Result negative. 6,095 flies failed to infect the monkey. From February 28th till April 7th monkey 633 was

examined daily—trypanosomes were never found in its blood. On March 27th 2 c.c. of Monkey 633's blood was injected subcutaneously into White Rat 720. The result of this injection was negative.

Experiment 641. Monkey.

Kibanga flies.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Feb. 27	480		Mar. 20	190	—
" 28	350	—	" 21	400	—
Mar. 1	300		" 22	575	—
" 3	400	—	" 25	570	—
" 6	375	—	" 27	600	—
" 8	475	—	" 28	580	—
" 9	350	—	" 29	130	—
" 10	475	—	" 30	500	—
" 14	650	—	" 31	750	—
" 15	300	—	Apr. 1-22	—	—

Remarks.—Result negative. 8,450 flies were fed on this monkey without infecting it. The monkey's blood was examined daily up to April 22nd.

Experiment 592. Monkey.

To ascertain if *Glossina palpalis* caught on the Islands are capable of giving rise to Sleeping Sickness in healthy monkeys.

Nsadzi Island.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Feb. 4	450		Feb. 9	335	—
" 5	450		" 10	280	—
" 6	275		Feb. 13- Mar. 3	—	—
" 7	300				
" 8	410	—			

Remarks.—Result negative. From March 7—March 27 monkey 592 was examined bi-weekly and no trypanosomes were ever detected in its blood. In this experiment 2,500 flies failed to infect a monkey.

Experiment 591. Monkey.

Nsadzi Island.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Feb. 2	300		Feb. 8	410	
" 3	465		" 9	335	
" 4	450		" 10	280	
" 5	450		Feb. 13-	} —	—
" 6	275		Mar. 1		
" 7	300				

Remarks.—Result negative. From March 1—March 27 monkey 591 was examined bi-weekly and trypanosomes were never found in its blood. In this experiment 3,265 flies failed to cause an infection.

Experiment 557. Monkey.

Kimi Island.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Jan. 22	590		Jan. 28	64	
" 23	650		" 29	750	
" 24	550		Jan. 31-	} —	—
" 25	590		Feb. 21		
" 26	540		Mar. 14	—	—
" 27	590				

Remarks.—Result negative. 4,324 flies failed to infect monkey 557. From February 24—March 14 monkey was examined bi-weekly. No trypanosomes were ever seen.

Experiment 558. Monkey.

Kimi Island.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Jan. 22	540		Jan. 28	60	
" 23	600		" 29	750	
" 24	610		Jan. 31-	} —	—
" 25	510		Feb. 21		
" 26	540		Mar. 14	—	—
" 27	510				

Remarks.—Result negative. Monkey 558 was examined bi-weekly from February 24—March 14 with negative results. Here 4,120 flies failed to cause an infection.

The above experiments are summarised in Table I.

Table I.

Date.	Experi- ment.	Place.	No. of flies put on.	Result.	Remarks.
1910.					
Aug. 8-15 ...	21	Kibanga	44	+	Flies caught on mainland.
" 16-Oct. 19	48	"	3,220	—	
Sept. 1-Dec. 31	122	"	6,625	—	
" 25-Oct. 1	198	Nsanga	2,390	—	
" 28-Oct. 1	199	"	1,940	—	
Oct. 2-6 ...	208	"	2,090	—	
" 2-6 ...	209	"	1,780	+	
" 7-13 ...	223	"	2,160	+	
" 7-13 ...	224	"	2,580	—	
" 22-Nov. 7	289	Bukafu	1,750	—	
" 22-Nov. 7	290	"	1,750	—	Flies caught on islands.
Nov. 16-21 ...	321	Kibanga	850	+	
1911.					
Jan. 3-Feb. 22	478	"	6,279	—	
Feb. 13-20 ...	597	"	3,075	+	
" 23-Mar. 16	633	"	6,095	—	
" 27-Mar. 31	641	"	8,450	—	
Jan. 22-29 ...	557	Kimi Island	4,324	—	
" 22-29 ...	558	"	4,120	—	
Feb. 2-10 ...	591	Nsadzi Island	3,265	—	
" 4-10 ...	592	"	2,500	—	

Table II.

Date.	No. of flies put on.	No. of infections.	Average No. of flies to cause infection.
1910-11.			
4 months, August to November.	22,784	4	5,696
4 months, December to March.	28,294	1	28,294

In Table II. the results obtained with flies caught on the mainland during the four months August to November, 1910, are compared with those for the four months December, 1910, to March, 1911.

When these results are compared with those reported by the Sleeping Sickness Commission of the Royal Society, 1908-09, the point which is most striking is the large number of negative experiments now recorded and the great increase in the number of flies necessary to infect a healthy monkey with *Trypanosoma gambiense* by feeding wild *Glossina palpalis* upon it. When the 1908-9 Commission's experiments were carried out—from November, 1908, until September, 1909—about 500 flies caught on the mainland were usually sufficient to infect a monkey. We now find from observations made during the period August, 1910, to

March, 1911, that some 10,000 are required, and that during the latter half of that time 28,294 flies were put upon susceptible monkeys and caused only one infection. These facts seem to indicate that the infectivity of Lake-shore *Glossina palpalis* is gradually becoming less.

There are, however, still a few infected flies to be found. Where they derive their infectivity it is impossible to say definitely.

The mainland population was removed from within two miles of the Lake shore early in 1908, so that it is now about three years since their removal. In spite of the prohibition, however, a few natives still frequent the Lake shore and it does not seem to be unlikely that they may be the source of infection. When experiments were being conducted at Bukafu several instances of natives frequenting the Lake shore came to our notice. In January, 1911, natives were seen with a canoe in Buka Bay. P. W. Cooper, Esq., District Commissioner, informs us that four natives were captured recently in this district. Such natives, some of whom are Islanders, have probably spent the whole of their lives on the Lake, so that the chances that some of them suffer from Sleeping Sickness seems fairly great. Unfortunately we could examine only one of the persons caught. Dr. R. E. McConnell, M.D., while acting as Medical Officer, Kampala, kindly injected this man's blood into a monkey. The result was negative.

It is, then, certain that *Glossina palpalis* still have access to human beings, some of whom are likely to harbour *Trypanosoma gambiense*, and this would appear to be the most probable explanation of the flies still retaining their infectivity.

Fly boys and canoe men employed collecting flies and their pupæ have been kept under constant observation. Blood from each of them has been injected monthly into susceptible monkeys. The results have been invariably negative. In two experiments clean laboratory-bred *Glossina palpalis* were fed once upon a canoe man and subsequently upon normal monkeys which did not become infected. All the flies dissected were negative for flagellates.

The examination of Lake-shore animals, which forms the subject of a separate paper, has as yet failed to show that they become naturally infected with *Trypanosoma gambiense*.

Conclusions.

1. *Glossina palpalis* caught on the shores of the Victoria Nyanza still remained capable of infecting monkeys with *Trypanosoma gambiense* up to February, 1911—that is, three years after the removal of the population from within two miles of the Lake.

2. The infectivity of the Lake-shore *Glossina palpalis* is diminishing.

3. The presence of natives at the Lake, in spite of prohibition, may be sufficient in itself, without the existence of any other reservoir, to account for the continued infectivity of the flies.

4. The results recorded in this paper, when taken in conjunction with the examination of wild Lake-shore animals and antelope artificially infected, with *Trypanosoma gambiense*, seem to point to the dying out of the infectivity of Lake-shore *Glossina palpalis*.

10.—VARIOUS EXPERIMENTS.

By Capt. A. D. FRASER, R.A.M.C., and Dr. H. L. DUKE.

The experiments described in this paper deal with a variety of subjects not dealt with in other papers.

A.—HEREDITARY TRANSMISSION.

Thirteen experiments were carried out to ascertain if flagellates in the fly were transmitted hereditarily.

853 laboratory-bred *G. palpalis* were fed for periods of 30 days or more upon normal monkeys. These monkeys were examined regularly. All remained healthy.

529 of these flies were dissected on different days throughout the experiments and no flagellates were ever found.

In addition to the above experiments in which flies were fed throughout upon normal monkeys, six other experiments in which the flies were fed for a few days on animals known not to be infected, *e.g.* healthy man, &c., before being transferred to healthy monkeys may be taken as additional evidence. In these, 266 flies were used, 216 of which were dissected and found negative to flagellates, while the monkeys fed upon remained healthy.

Remarks.—1,119 flies were fed on normal monkeys for thirty days or more. All the monkeys remained healthy. 745 flies were dissected and in none were any flagellates found.

B.—LONGEVITY OF LABORATORY-BRED *G. palpalis*.

In the following three experiments the daily feeding of the flies was continued until their natural death. In Experiments 1 and 5 the sexes were mingled, while in Experiments 326 and 327 the sexes were kept separate.

Experiment Number.	Number of flies alive on:—											
	1st day.		100th day.		125th day.		150th day.		160th day.		185th day.	
	Males.	Females.	Males.	Females.	Males.	Females.	Males.	Females.	Males.	Females.	Males.	Females.
1 	82	88	6	9	1	2	—	1	—	1	—	—
5 	25	19	5	8	—	4	—	3	—	2	—	1
326 (Males only)...	24		3		1*							
327 (Females only)	22		3		1*							

* Still alive on the 147th day.

Remarks.—On one occasion a fly—a female—survived in captivity until the 185th day. Experiment 327 served to show that out of 22 females employed none showed parthenogenesis.

C.—CAN LABORATORY-BRED *G. palpalis* ACQUIRE *T. grayi* BY CONTAMINATED FEEDING?

On November 16, 1910, 230 Lake-shore *G. palpalis* were placed over a dish containing a little water, after feeding on a normal animal, in order to collect their fæces. On November 17, after feeding on a normal fowl the fæces of the same 230 flies were again collected and the whole emulsion thus obtained smeared on the shaven belly of Monkey, Experiment 328. On November 18 and 21, the process was repeated with 150 and 110 flies respectively. Clean laboratory-bred flies were then fed upon the monkey, being placed upon the smeared surface.

Date.	Day of experiment.	Procedure.	No. of flies dissected.	No. shewing flagellates.
1910. Nov. 17 ...	—	45 clean laboratory-bred flies of Experiments 326 and 327 fed on monkey, Experiment 328.	—	—
Nov. 18, 1910, to Mar. 22 1911.	1-125	Fed daily on monkey, 328.	42	—

Remarks.—Result negative. On March 22, two flies remained alive. Monkey remained healthy.

Experiment 113.

In this experiment, Monkey, Experiment 114, upon which the flies were fed was smeared on the shaved abdomen on August 31 with flagellates from five Kibanga flies which showed infection with *T. grayi*.

Date.	Day of experiment.	Procedure.	No. of flies dissected.	No. of flies showing flagellates.
1910. Sept. 1 to Oct. 17.	1-47	57 clean laboratory-bred <i>G. palpalis</i> of Experiment 113 fed daily on monkey, Experiment 114.	—	—
Oct. 18 ...	48	Starved. Remaining flies, 42, dissected.	56	—

Remarks.—Result negative. On September 27th, monkey 114 was smeared on belly with an emulsion of wild fly fæces. Monkey remained healthy.

Experiment 188.

Every fly used in this experiment, previous to feeding on the monkey, had its proboscis thoroughly smeared in the emulsion of wild fly faeces. It was then fed upon Monkey Experiment 114 of the previous experiment. Seventeen flies were thus treated, and subsequently fed upon Monkey Experiment 114 for 20 days. Sixteen were dissected during the experiment and none contained flagellates.

Remarks.—In these three experiments, where it was attempted to infect laboratory-bred *G. palpalis* with *T. grayi* by contaminated feeding, a total of 114 flies were dissected and in none were any flagellates found.

D.—EXPERIMENT 191.

In this experiment the clear fluid which exudes from the anus of *Glossina palpalis* after a feed was injected subcutaneously into a healthy monkey to ascertain whether *T. gambiense* could be transmitted from sick to healthy by this means. The flies chosen were those of Experiment 50, a positive *T. gambiense* "cycle." After the monkey experiment 50 became infected the flies were allowed to continue their daily feeding upon it, the fluid being collected in a pipette and injected immediately. Injection was in this way performed daily from September 27—October 15.

The monkey 191 was examined bi-weekly up till November 18, and remained uninfected.

Microscopical examination of this fluid never revealed flagellates on the few occasions on which it was examined.

E.—EXPERIMENT 250.

Flies caught at Kibanga and placed in a cage together with a frog were seen to feed readily. Flies bred in the laboratory were also observed to feed upon frogs. On one occasion the frog, irritated by handling, exuded a viscid substance from its back which caught and eventually killed the flies which settled upon it. In a short time, the secretion having dried, the remaining flies were seen to feed unconcernedly.

In a cage where frog and flies were left overnight the former on dissection showed numerous wings of *G. palpalis* in its stomach.

F.—DISSECTION OF BITING FLIES OTHER THAN *G. palpalis*.

Species of fly.	Number dissected.	Number positive to crithidia.	Microfilaria.
<i>Tabanus secedens</i>	128	5	2
<i>Tabanus insignis</i>	3	—	—
<i>Tabanus thoracinus</i>	168	6	—
<i>Haematopota similis</i>	2	—	—
<i>Chrysops funebris</i>	35	1	—

Remarks.—On two occasions, once with *Chrysops funebris* and once with *Tabanus secedens*, the crithidia found were injected

into white rats, in each case with negative results. In one of the chrysops dissected an opaque white cyst was seen in the gut just behind the attachment of the Malpighian tubes. This cyst was easily visible to the naked eye, being about the size of a pin's-head. Under pressure of the coverglass the cyst ruptured, and a number of oval bodies were liberated which again set free still smaller rod-shape bodies.

As seen in a dry film stained with Giemsa these oval bodies were seen to be navicelliform or barrel-shaped spores with a clear very faintly staining coat. At each end was a deeply staining plug. Owing to poor fixation, details as to the outline of the spores could not be satisfactorily made out, but there appeared to be short appendages at the poles. The spores and cyst resembled those of a gregarine. On another occasion a gregarine was seen in the gut of a *Tabanus secedens*.

11.—EXPERIMENTS DEVISED AND CARRIED OUT BY DR. R. VAN SOMEREN.

1. Treatment by arsenophenylglycin of *Trypanosoma gambiense* infected monkeys.

The following table gives the quantity of the drug which was given, the method of administration, the number of days which elapsed after trypanosomes first appeared in the monkey's blood before treatment, and the number of days the monkey survived the injection.

Table.—Treatment of Trypanosome-infected Monkeys by
Arsenophenylglycin.

Experi- ment.	Dose in grammes.	Method of administration.	No. of days after trypanosomes appeared when treatment was begun.	No. of days monkey survived treatment.
63	·5	?	2	178
64	·5	?	?	41
81	·5	Intramuscular injection.	4	3
132	·5	?	0	32
133	·5	?	0	7

Remarks.—Five monkeys, whose body weights are not recorded, were treated with arsenophenylglycin—5 grammes. The average number of days which they survived the treatment works out at 52 days. Monkey Experiment 81 is noted to be “very small and thin.” It would appear that the dose used was a very large one for a monkey of average weight. The method of administration, when this is not recorded, was most probably by subcutaneous injection.

2. To ascertain if *Stomoxys* can act as a carrier of trypanosomiasis.

Two experiments were carried out. In one, 100 *Stomoxys* were fed from the 1-5 day of the experiment on a *Trypanosoma brucei* infected monkey. They were then, after having been starved for 24 hours, fed on a healthy monkey for one day when the note "most *Stomoxys* dead" was made. The healthy monkey was examined bi-weekly for six weeks. Trypanosomes were never found in its blood. In a second experiment the trypanosome used was probably *T. gambiense*. The result was again negative.

Remarks.—No definite conclusion can be arrived at from the above experiments. Apparently few flies were alive when the flies were transferred to the healthy monkey. None of the flies appear to have been dissected.

3. To ascertain if the pupæ of *Glossina palpalis* are destroyed by submerging them in water.

Pupæ collected on the Lake shore were placed in a dish containing water to the depth of one inch.

Result.—Of 64 pupæ so treated for 11 days, 30 hatched out during the 10 days following their removal.

12.—PROGRESS REPORT ON INVESTIGATIONS INTO THE BIONOMICS OF *GLOSSINA PALPALIS*, JULY 27, 1910, TO AUGUST 5, 1911.

By G. D. H. CARPENTER, B.A., B.M., B.Ch. (Oxon.), F.E.S.
(Uganda Medical Service.)

(Plates 26-29.)

PRELIMINARY.

The investigations reported in this paper were conducted from July 27, 1910—August 5, 1911.

It must be understood throughout that they refer only to *Glossina palpalis*, and that when the term *Glossina* is used for brevity it refers only to that species.

They were mainly concerned with the following points suggested by the Tropical Diseases Committee of the Royal Society:—

1. Length of life of the fly.
2. Duration of infectivity of the Lake-shore fly.
3. Natural enemies of the fly in all its stages.
4. Breeding grounds.
5. Food supply.

In addition, attention has been directed to—

6. Relation between bacilli and trypanosomes in the gut of the fly.
7. The contents of the gut of freshly-bred flies.
8. The varying sex proportion.
9. Tsetse-fuges.
10. The tolerance of pupæ to various conditions.

A summary of conclusions arrived at is given at the end of the Report.

THE PLACES SELECTED.

From July 27, 1910–February 11, 1911, inclusive I was at Jinja, Usoga, and after March 1, with the exception of a fortnight on Nsazi Island at the commencement of the month, I was on Damba Island.

Kerinya.—Near Jinja was a small peninsula named Kerinya, whose neck was narrowed, and along one side of which was untouched forest growth; on the other, papyrus reeds and swamp.

The former sheltered a few flies only, the latter none. The end of the peninsula was formed by a hill rising sharply some few hundred feet, and descending as sharply to a flat, open, grassy space continued almost to the water's edge, where there were trees and enough bushes to shelter fly.

This area was made the site for the first investigation into the length of life of the fly, for which it was very suitable, since there was probably no influx of flies from elsewhere; they being extremely few along the neck of the peninsula and on the coast line of the peninsula generally, owing to its being mostly fringed with reeds and papyrus.

Hence there was probably little dilution of the numbers of the local flies by an influx from surrounding parts.

Nsazi Island.—This is a small islet some three miles long and less than half that width, lying south of Entebbe.

At only one place along its coast were flies at all numerous; here was a strip of fine sand backed by the abrupt face of a small cliff, at the foot of which was a narrow, but dense, strip of forest growth; the tops of the trees being on a level with the top of the cliff.

Damba Island.—Nsazi Island not proving altogether suitable I moved to Damba, a large island south-east of Entebbe, lying on the Equator. At one place along the shore flies are extremely numerous.

Here the beach is of pebbles, backed by a strip of dense forest growth at the foot of an outcropping rock-face some twenty feet high.

At the edge of this strip there are places where the coarser pebbles are mixed with sand—these appear to be the most suitable places for *pupae*.

LENGTH OF LIFE OF FLY.

Method adopted.—The method adopted was to mark large numbers of flies, liberate them, and ascertain for how long afterwards such marked specimens could be caught at the same spot, the fly-boys catching flies daily, except Sundays, and I examining each one.

A fly was marked by amputation of a leg through the centre of either femur or tibia; thus twelve different markings are possible.

No wild fly is ever caught so mutilated; the natural loss of part of a leg always occurs through a joint.

The following table shows the number of flies marked:—

Jinja Marking Experiment.

Table I.

Date.	No. of mark.	♂	♀	*Total.
July 27-Aug. 2 ...	I.	475	542	1,017
Aug. 3-Aug. 9 ...	II.	326	379	705
Aug. 10-Aug. 16 ...	III.	446	576	1,022
Aug. 18-Aug. 27 ...	IV.	599	551	1,150
Aug. 29-Sept. 6 ...	V.	574	545	1,119
Sept. 7-Sept. 17 ...	VI.	532	555	1,087
Sept. 19-Sept. 28 ...	VII.	471	590	1,061
Sept. 29-Oct. 15 ...	VIII.	482	500	982
Total ...		3,905	4,238	8,143

Of these eight thousand flies marked, those that were subsequently caught each day were noted and again liberated.

The following table shows the result:—

Table II.

Marking ...	1	2	3	4	5	6	7	8
Sex ...	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀
Date.								
Aug. 3 ...	15 11							
" 4 ...	1 2							
" 5 ...	3 2							
" 6 ...	2 7							
" 8 ...	3 5							
" 9 ...	1							
" 10 ...	2 14	7 7						
" 11 ...	1 2	3						
" 12 ...	2 1	2 2						
" 13 ...	1 1	1 1						
" 15 ...	2 12	3 10						
" 16 ...	9 10	5 7						
" 18 ...	7 5	2 6	7 3					
" 19 ...	2 2	2 3	7 10					
" 20 ...	2 4	2 4	2 8					
" 22 ...	2 1	1	6 6					
" 23 ...	1 3	4 1	4 3					
" 24 ...	1	2	6 2					
" 25 ...	1 7	1 2	2 2					
" 26 ...	1	1 2	4 4					
" 27 ...	6	2 4	1					
" 29 ...	1		1 1					
" 30 ...	9 3	1 1	2 3	6 8				
" 31 ...	2 3	1 1	1 5	7 11				
Sept. 1 ...	3	1 2	2	5 6				
" 2 ...	5 3	1	2	2 1				
" 3 ...	3 3	2 1	4 1	3 5				
" 5 ...	1	1 1	2 1	1 7				

Table II.—*continued.*

Marking ... Sex ...	1		2		3		4		5		6		7		8	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Date. ●																
Sept. 6 ...	2	4	3			1										
" 7 ...				2	3		1	5	17	21						
" 8 ...	1		1			1	2		4	2						
" 9 ...	1	2			1	1	1	3	1	3						
" 10 ...		2			4	1	4	1	4	5						
" 12 ...	1						2		2	2						
" 13 ...	5		1		2			3	8							
" 14 ...		1			1		1	2	6	3						
" 15 ...		2	1	1	1	1	2	2	3	2						
" 16 ...	1	2	2		7	2	3	2	10	7						
" 17 ...		2			4	2	1	5	6	4						
" 19 ...	1								5	2	10	2				
" 20 ...		1		1	1	2			9	3	21	5				
" 21 ...	1	1	3		1	1	1	2	2	3	13	6				
" 22 ...	1	1	4		1		2	6	2	7	10	11				
" 23 ...		1							1	3	3	3				
" 24 ...	2	1			1		2		5	2	4	2				
" 26 ...											1	2				
" 27 ...		1	1				1				6	7				
" 28 ...			1		1		1		4		3	2				
" 29 ...	1				1				1		1					
" 30 ...	1								1	1	1					
Oct. 1 ...		1														
" 3 ...					2		1	1	2		3	1				
" 4 ...		2					1	1								
" 5 ...	1							1			6	3				
" 6 ...		2	1						1	4	3					
" 7 ...											3					
" 8 ...	1								1		4	2				
" 10 ...			2		1		1		2	1		3				
" 11 ...							1				2					
" 12 ...											1					
" 13 ...	1				1		1				1	2				
" 15 ...									1		1					
" 17 ...		2									2			3		
" 18 ...										1		2	1	1		
" 19 ...	1										1			2		
" 20 ...								2				1				
" 24 ...		2	1		1	1			2		1	3		2		
" 25 ...	3	1									2					
" 26 ...									2	1		2	1	1		
" 27 ...									1							
" 29 ...											1	1		1		
" 31 ...		1														
Nov. 4 ...		1											2		3	1
" 5 ...							1						1			
" 8 ...															2	
" 9 ...	2		1												1	
" 10 ...											1		1		1	2
" 11 ...														1	1	2
" 12 ...		1													1	2
" 14 ...															1	1
" 16 ...													1	2	1	2
" 18 ...																1
" 22 ...			1													
" 25 ...														1		
" 26 ...													1			

Table II.—continued.

Marking ... Sex ...	1		2		3		4		5		6		7		8	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Date.																
Dec. 3 ...											1		1			
" 5 ...																
" 7 ...													1		1	
" 9 ...		1													1	
" 15 ...															1	
" 16 ...					1								1		1	
" 17 ...													1			
" 19 ...		1													1	
" 20 ...															1	
" 24 ...															1	
" 28 ...															1	
" 29 ...		1														
" 30 ...					1											
" 31 ...									1							
Jan. 6 ...									1							
" 7 ...		1														
" 13 ...									1							
" 23 ...		1														
" 31 ...		1														
Feb. 1 ...									1							
" 7 ...									1							
" 8 ...	} Flies caught daily but no marked ones taken.															
" 9 ...																
" 10 ...																
" 11 ...																

After the end of January flies had become so reduced in numbers that it was with difficulty that the fly-boys could catch two dozen in three hours. It will be noticed, from the table, that flies of the first marking survived almost up to the last date on which a marked fly was caught; and that of the last marking (which was done when the number of flies that were caught had very considerably decreased and they were presumably dying in quantities) very few of the flies were retaken.

The fly caught on January 31st had been marked on one of the days July 27th-August 2nd inclusive; its life at a minimum must have been 182 days, and it is of course impossible to say for how long it had been hatched before July 27. The chances are, however, that it was a freshly hatched fly when it was marked.

This fly was a female; a male of the first marking was last retaken on December 29th, giving a life of minimum duration of 149 days.

As the diminution in number of flies corresponded with the onset of hot dry weather, charts were prepared showing the relation for each month between meteorological conditions and the number of flies. For the meteorological data I am indebted to Dr. J. M. Collyns, Medical Officer at Jinja.

The temperature charted each day is the mean of three readings of the dry bulb thermometer taken daily at 7 a.m., 2 p.m., and 9 p.m. No hygrometer records were obtainable, but the relative

humidity is indicated daily by the difference between the mean readings of wet- and dry-bulb thermometers. Inasmuch as a high figure indicates a low degree of humidity, the "humidity" curve is inverted on the chart. The rainfall was estimated daily at 7 a.m.



Fig. 1.—Chart showing an average for each month of the daily records given in Fig 1. The rainfall is the total for each month. DESCRIPTION OF SCALES.—Rainfall—Total rainfall per month in inches. Temperature—Average daily temperature in deg. Fahr., as result of readings at 7 a.m., 2 p.m., and 9 p.m. Humidity—Average daily difference in deg. Fahr. between wet and dry bulb thermometers. Flies—Average daily catch of flies per hour (see 8 p. 100). Sun—Average hours of daily sunshine.

The "fly" figure on the chart indicates daily the average catch of flies per fly-boy per hour. In order to eliminate error due to laziness on the part of the fly-boys, I was present each day for the whole time to see that each boy was working to the best of his ability.

As on some days flies were not caught the curve is joined up in places by dotted lines. In order to make the other curves strictly comparable they are corrected by dotted lines between the same points.

A summary of these results, showing the average figures for one day of each month, and the total rainfall per month, brings out in a striking manner the effect of meteorological conditions upon the number of flies (*see* Fig. 1). It will be seen that the humidity curve steadily descended during the 6 months, with the exception of a slight recovery in December, and that the fly curve varies directly with it.

A high temperature has the opposite relation; the fly curve varying inversely as the temperature curve. On any individual day, however, the day's catch depended rather directly upon the height of the Temperature, for on a cool day flies do not appear on the wing in such quantities.

Thus there is often a fair correspondence between the fly and temperature curves in an individual month (*e.g.*, August); and the effect of temperature, sun, and humidity factors may be seen on individual days, *e.g.*, August 22-24, 25; September 16, 26-27; October 11, 19, 22, 28; November 19, 24; December 10, 12, 13, 23.

These factors, with rainfall, should all be considered together; sometimes one, sometimes another, appearing dominant.

The marked irregularity of the fly curve in December and January, when the numbers were declining, is noteworthy.

A decrease in the relative humidity of the atmosphere is therefore responsible for a large drop in the number of flies; and taking into account the marking experiments it appears that flies can live several months, but are killed off in large numbers at the onset of dry weather.

In order to confirm this result, on arrival at Damba Island in the wet season, I again marked large numbers of flies.

Damba Marking Experiment.

Table III.

Date.				Marking.	♂	♀	Total.
March	17-22	...	...	I.	1,169	237	1,406
	27-31	...	...	II.	682	219	901
"	4-8	...	...	III.	745	228	973
"	10-14	...	...	IV.	660	198	858
"	20-22	...	...	V.	387	145	532
Totals				...	3,643	1,027	4,670

A number of *bred* flies were also marked and liberated.

April 10-14	...	...	IX.	...	278
April 20-May 3	...	...	VIII.	...	668
May 4-15	...	...	VII.	...	175

Total 1,121

Between March 17 and April 22, 4,670 flies were caught, marked, and liberated; and between April 10 and May 15 1,121 bred flies were differently marked and released.

The months of March, April, and May were very wet. In June there was much less rain, and July was hot and brilliantly fine.

After July 2nd no rain fell until 15th and 16th, when there was a very little in early morning; again on 22nd and 23rd; steady rain for a few hours on the 29th; a little early on 31st; on the night of August 2nd a little; and then a spell of brilliantly fine and hot weather.

On July 12th I visited the place where the flies had been marked, and promised the fly-boys a reward for each marked fly they caught, but none were taken.

On July 14th I examined all the flies caught, and on subsequent days, but no marked one was retaken on any day.

July 14th ... 451 flies caught and examined.

„ 31st ... 295 „ „ „

August 2nd ... 419 „ „ „

„ 4th ... 514 „ „ „

Taking into account July 12th, about 2,000 flies were examined in five days, without finding one of the 6,000 previously marked 4-3 months ago.

The dry weather of July and August would appear to be responsible for killing off these flies.

Conclusions.

1. Flies can live for 182 days in the case of females; males for 149 days. These are minimum times.

2. A spell of dry weather is fatal to flies which have been living several months; and

3. The decrease in the relative humidity of the atmosphere is responsible for the decrease in the numbers of flies noticed at the onset of dry weather.

II.—DURATION OF INFECTIVITY.

The following experiment on Damba Island proves the flies to be still infective.

The monkey was a male, and showed no trypanosomes in its blood.

Table IV.

Date.	Flies fed on monkey.			Blood.
	♂	♀	Total.	
1911.				
May 5 ...	226	61	287	—
" 6 ...	249	103	352	—
" 8 ...	190	56	246	—
" 12 ...				—
" 13 ...				Flagellating malarial organism.
" 15 ...				Many trypanosomes.

On May 8th the monkey appeared to have got a sunstroke as the result of being held in the sun: he was very sick, and unwell for some days, so was left alone. On the 15th, when a fresh batch of flies was being fed, the blood was examined and showed numerous trypanosomes. It appears, therefore, that 885 flies were sufficient to cause infection.

The sources from which the flies derive trypanosomes on the island, since the date when the last islander was removed, must be either the local fauna, or visitors.

The canoe men from Mpumu, who come regularly for pupæ, are examined monthly at Mpumu, and I am assured by Dr. Duke that no case of infection has been found. I examined in the same way myself and the fly boys, blood from each of us being injected into a monkey subcutaneously: the monkey's blood was examined weekly for a month without showing trypanosomes, and the monkey remains healthy to this day.

The natural sources of infection on the island may be found amongst the local fauna. The only mammals I know of on the island are monkeys, cats, otters, bats, shrews, small rodents, hippopotami, and the tragelaph or "marsh buck."

Of birds, the cormorants and darters which sit on the rocks a little way from the shore, are especially likely to be fed upon by *Glossina*.

Crocodiles are very scarce and seldom seen in the neighbourhood: the *Varanus* lizard (Monitor or Nswa-swa) is extremely common.

Conclusion.

A year and ten months after the removal of the last inhabitant from Damba Island, 885 flies were sufficient to infect a monkey.

III.—NATURAL ENEMIES OF GLOSSINA.

A. Of the Larva.

The peculiar method of reproduction of *Glossina* leaves little scope for enemies of the larva, whose extracorporeal life is restricted to a few minutes.

Nevertheless, it is possible that some creature may be found which would snap up the larva, or deposit eggs in it, during the brief time it is exposed before it has buried itself. Accordingly I have spent many hours watching at spots especially selected by *Glossina* for larvi-positing, but have never seen anything happen to the larva.

On one occasion, on arrival at the spot, I saw a large black ant (*Paltothyreus tarsatus**) carrying off in its mandibles a freshly extruded larva.

This ant is of slow habit, and wanders over the surface of the ground searching for food in the crannies under stones.

I have often seen it wandering about whilst the flies were larvi-positing, and on several occasions have placed a wriggling larva in front of one of them, which immediately seized the larva and gave it several nips with its large mandibles, eventually carrying it off to the nest.

The ants seemed so thoroughly familiar with the larvæ that I think they must find, and consume, a certain number. I have endeavoured to ascertain whether there may not be a Hymenopterous parasite which would lay its eggs in the larva while still in utero.

To this end females containing larvæ full grown, or nearly so, were caged until the larvæ were extruded.

But very few full grown larvæ could be obtained, owing to the flies dying or aborting: so that from a large number of flies only 50 pupæ were obtained, from none of which were any parasites reared.

B. Of the Pupa.

I have obtained no evidence that Vertebrates destroy the pupæ.

Inasmuch as pupæ are often found collected in great numbers at favourite spots, any bird or animal which preyed upon them would naturally look for them in such places, and in so doing would leave traces; but I have never seen any evidence that the loose gravel, in which pupæ are found collected, had been disturbed.

I have not succeeded in breeding any parasites on Damba Island from 5,600 pupæ kept in wooden boxes closed by glass lids.

It may be worth noting here that at the place on the lake shore near Mpumu, where pupæ were formerly collected for laboratory purposes, I found on a brief visit, numbers of pupæ with a small round hole indicating the exit of a parasite.

From another species of Dipterous pupa obtained in the same locality a species of Hymenopterous parasite (*Chalcididæ*) emerged through a small round hole precisely similar to that in the *Glossina* pupæ.

This hole is usually at about the centre of the pupa. I am informed by Dr. Duke, now in charge of the laboratory at Mpumu, that there has been such a diminution of flies in this locality that it is no longer worth while to collect pupæ from there, and it is possible that the parasite may have been partly responsible for this reduction.

* Identified by Mr. C. C. Gowdey, Government Entomologist at Entebbe.

Fig. 2, A, indicates the appearance of this small round hole made when the perfect parasite bites its way out through the shell. On holding such a pupa up to the light it is seen that the whole contents have been devoured. Of such pupæ two only have been obtained on Damba, out of many hundreds of normal ones, although the fly boys have been told to be on the look-out for them. But several have been found showing that there is some insect which bites a hole from the outside and then devours as much of the contents as it can reach.



FIG. 2.—Outlines of two pupæ (copied from Austen's "Handbook") $\times 6$. In A is seen the small hole made by exit of a parasite. In B the irregular hole made from outside by an enemy which devoured the contents.

The hole made thus is irregular, with jagged edges, and is often at one end of the pupa, at the base of the pair of processes. This is shown in Fig. 2, B. On holding such pupæ up to the light some may be seen to be opaque at the other end, indicating that the enemy has not been able to reach the entire contents. Sometimes the larva appears to have been attacked before the skin has hardened to the pupal condition; for the fly boys have brought me several wrinkled, shrivelled larval skins, clearly showing the segmentation and preserving the larval shape, at the end of which a similar hole had been bitten, and the contents devoured.

I am unable to state what is the insect which does this. Beetles and cockroaches, of several species, which are found buried together with the pupæ, have been enclosed in a box with pupæ and the material in which they were found, but none have ever attacked the pupæ.

Very important enemies of pupæ in England are woodlice (*Oniscoida*). But the only species I have found associated with *Glossina* pupa are small and feeble, and when enclosed in boxes with pupæ always died. Amongst the Myriapoda the Chilognatha (*Millipedes*) come under suspicion as likely to devour pupæ. At Jinja I found a small species of Gulidæ amongst pebbles and sticks where I had found a few pupæ. This, being enclosed in a box with a pupa, bit a large hole and devoured part of the contents and did the same to a second pupa. But I do not consider this of much importance, for the conditions favourable for pupæ are not so for Gulidæ, which require more moisture than do *Glossina* pupæ.

C. Of the Freshly Hatched Fly.

The freshly hatched, soft, and comparatively helpless fly may be expected to fall an easy victim until its wings have expanded and hardened for use. But they are very alert and run very quickly when alarmed. I have several times seen them resting on pebbles on the breeding ground, while the wings filled out, and if an ant came near enough to be dangerous they quickly sidled away a few steps, or ran vigorously for a short distance.

The wings and integument are hardened about half an hour after the fly has emerged from the pupa, but, when driven, it can take a brief flight much before that time.

D. Of the Perfect Fly.

VERTEBRATES.

I. *Bats*.—In the fly area at Damba there is a cave in the rock face wherein many bats hang during daylight. Five of these, shot early one morning, were examined, but their stomachs were all empty. The bat, being a nocturnal feeder, is hardly likely to prove an enemy of *Glossina*.

II. *Birds*.—I have examined, with negative results, the stomach contents of 26 bee eaters, belonging to three species, though the majority were of one. These were all shot whilst feeding in the fly area; the stomach contents were mostly Hymenoptera and Dragon flies.

Of other birds, 26 specimens of various insectivorous species have been examined with negative results. These included insectivorous kingfishers, fly-catchers, warblers, weaver birds, a shrike, and cuckoos. A stone curlew, which was shot on Nsazi Island, having been seen running about over the pupa ground, proved to have eaten only a small frog and a cricket.

III. *Lizards*.—I found it impossible to catch lizards, but have shot 5 of a large, tree-frequenting species, whose male is bright blue, which comes down to feed among low herbage. They, however, appear to feed almost entirely on small ants.

IV. *Frogs*.—53 of a species of green tree frog have been examined; they sit on herbage by day, and were taken in the fly area. They feed chiefly on spiders and sluggish insects such as Hemiptera, and none had eaten *Glossina*. This was to be expected as these frogs do not feed in the hot part of the day when *Glossinae* are about.

INSECTS.

I. *Orthoptera*. *Family Mantidæ*.—I have never seen a mantis actually catch a *Glossina*, but at Jinja there was a species* (*Pseudocreobotra ocellata*, P. de B.) which sat on the flower-heads of sunflowers in the fly area, where it was admirably concealed by its green and yellow colouring, and caught any insect which flew within range or settled on the leaves and flower heads.

* Identified by the Entomological Research Committee.

Now Glossinæ very often settled on sunflowers, and there is every reason for supposing that they must fall occasional victims; this can, however, only be a matter of chance.

The mantis readily devoured Glossinæ offered to it.

II. *Neuroptera odonata*. Family *Libellulidæ*.—An extremely common Dragon fly of this family is *Cacergates leucosticta*, Burm.* I have several times seen specimens chasing Glossinæ, and heard them make darts at specimens buzzing under the brim of my hat. An intelligent fly-boy brought me one which he had seen catch and devour a Glossina, after which he netted it.

IV. A. *Hymenoptera parasitica*.—Once at Jinja a fly was caught which appeared to have a parasitic larva affixed to the under surface of the abdomen. Unfortunately whilst I was looking at this the fly escaped and I cannot speak with certainty.

B. *Hymenoptera aculeata*. Series *Fossoræ*, Family *Sphegidae*.—The fossorial wasps of the group *Bembecides* make Diptera their prey, and have a special fondness for *Tabanidæ*.

They excavate burrows in which the larva is fed upon flies provided fresh at frequent intervals, having been adroitly stung by the mother wasp. Owing to the necessity of frequently opening and closing the burrows, these are constructed in very light soil or sand, preferably with a loose surface through which a passage can easily be excavated, leading to a permanent chamber in the firmer substratum. Needless to say, the presence of much vegetation is not favourable, as the roots prevent burrows being made. In a favourable site many *Bembex* will make their nests, though they are strictly solitary wasps. Such a locality was found on Nsazi Island, where was a broad strip of sand, and a species† (*Bembex forcipata*, Hand.) nested there in numbers. I very soon saw one catch a Glossina off my clothes, and thereafter spent a considerable time noting their habits.

One morning I watched a *Bembex* provisioning its burrow. The wasp very quickly found there was an abundance of flies to be caught off my clothes, and I was able to watch it catch, sting, and carry into the burrow, one after the other, no less than 29 Glossinæ and two other flies in 3½ hours. The wasp flew round and round me, evidently picking out a suitable fly; it then pounced upon the fly, often catching and stinging it in the air, and carried it to the burrow between the femora of the middle pair of legs.

The burrow was then opened, the wasp scratching out the sand with the fore legs; the fly was carried in and deposited and the wasp came out again, filled up the entrance to the burrow with loose sand, and proceeded to catch another fly. At one time, within five minutes, four flies were caught and taken into the burrow.

When the wasp had finished and flown away I opened up the burrow, and found a full grown larva with 31 Glossinæ and two

* Identified by the Entomological Research Committee.

† Identified by Mr. C. C. Gowdey, Government Entomologist, Entebbe.

other flies. Of the Glossinæ, 21 were ♂ and 10 ♀, and of the females, eight were fat ones containing large larvæ, *i.e.*, 80 per cent. Now of the female flies caught by the fly boys that morning, only 22 per cent contained large larvæ. This shows that the *Bembex* selected the largest flies; as a matter of fact I am convinced that it expected to find full fed flies on me, as would have been the case had I been an unclothed animal, and that it was looking for such flies, heavy and easy to catch, when it flew round and round me before pouncing on a selected fly. The fact that it instinctively does this shows that it is a natural thing for it to prey upon Glossina, and that this was not an exceptional case.

The *Bembex* spends the night in the burrow underground, and does not emerge until the ground is warmed; the hotter the sun the greater its energy.

Although it is the rule that the wasp closes the burrow after every visit, yet it often does not do so, individual specimens appearing to be more forgetful; and a small Muscid fly of the genus *Idia* takes advantage of this lapse to enter the burrow and lay its own eggs there, so that its larvæ consume the flies intended for the *Bembex* larva, which may thus be starved by this indirect parasitism.

I have seen as many as four of these flies waiting around the burrow. Sometimes a fly will follow the *Bembex* into its burrow, literally tumbling head over heels in its anxiety to be quick, and coming out again before the wasp. In the majority of burrows which I explored there were several small pink Dipterous larvæ, presumably those of *Idia*, but they invariably died when attempts were made to rear them. Inasmuch as they were always found in a burrow with living larvæ they do not appear to attack it.

I am informed by the Secretary of the Entomological Research Committee that the species of *Idia* is a new one, and that this habit has not previously been recorded for the genus. The *Idia* must therefore be considered to be an enemy of the *Bembex*, and as such to play a part in the bionomics of Glossina.

It is, of course, only the female *Bembex* which is concerned with the burrows; the male flies up and down over a limited area, usually having a favourite resting-place on the ground, or flower heads.

Often when a female passes he rises swiftly off the ground to meet her, and congress occurs in the air during a brief flight.

The male is many times less numerous than the female. I am unable to state the duration of life of the larva. All the burrows that were opened and explored contained nearly full-grown larvæ.

One of these was kept and supplied with Glossinæ. On March 10, 5 p.m., it was given five flies, which were all consumed by next morning, and, at 7 a.m., five more were given, of which all but one were eaten by 2 p.m., so that, in 21 hours, nine Glossina had been devoured. Inasmuch as the larvæ shortly afterwards died, it is probable that this is an underestimate of the number of Glossinæ which would be accounted for by a larva.

Two other full-grown larvæ obtained spun cocoons soon after

I got them—firmly made, with grains of sand embodied in the outer layer; in shape an elongated oval $\frac{4}{5}$ inch long and $\frac{2}{5}$ inch broad. From one of these, which was constructed on March 13, the adult wasp emerged on July 2nd.

This *Bembex*, when it occurs, must be an important factor in keeping down the numbers of *Glossinæ*. In a site that is favourable for nesting numbers will congregate; each one constructs several burrows, in each of which is a larva ceaselessly devouring flies. Unfortunately the *Bembex* does not confine its attentions to *Glossinæ*; in the majority of burrows were other flies (often the parasitic *Idia* itself!), and I am inclined to think that it does not especially attack *Glossina* unless it can get full-fed specimens which are easily caught off the animal.

The readiness with which they came to catch *Glossinæ* off me, and, if there were none at that moment, flew off elsewhere and returned with other species of *Diptera*, supports this view.

The distribution of the *Bembex* must depend, to a great extent, upon whether there is a suitable breeding ground, which must be of such a nature that the wasp can easily excavate a burrow, and as easily fill it up again, leading to a permanent chamber for the larva in firmer soil. I found the burrows usually penetrated obliquely downwards some 4 to 5 inches.

It would not be difficult to increase the distribution of the *Bembex* by removing cocoons from the burrows and burying them elsewhere where similar favourable conditions obtained; but even then the distribution must be extremely limited.

I found at Kerinya, Jinja, another species of *Bembex* (*B. capensis**) of which there was a small colony of about six females and a male at one spot where the conditions were suitable for nesting. On one occasion I saw one carrying into its burrow a full-fed *Glossina*, whose swollen, shining red abdomen was unmistakable. I was unsuccessful in tracing this burrow; but found a few others, in one of which were remains of *Tabanus thoracinus* and *Chrysops brucei* amongst other flies. A larva which I obtained spun a cocoon similar to that of *B. forcipata*, but slightly pyriform. This *Bembex* was also visited by the parasitic *Idia*.

IV. C. *Hymenoptera aculeata*. Family *Formicidæ*.—Amongst ants there is a small and extremely plentiful species, making its nest in the ground, which has been found to attack *Glossina*. At Jinja, on several occasions flies were caught whose legs had been attacked by one of these ants whose head still remained affixed thereto by the mandibles, the body having become separated. One fly had the still living ant clinging to its leg. On Damba Island, whilst watching on the breeding ground, I saw a *Glossina* being dragged by a multitude of these ants into their burrow. These are a great nuisance in the camp, as they are able to get through small crannies, and often get into the boxes containing pupæ and cut up the freshly emerged flies. This may very possibly occur in nature: and they may also destroy resting

* Identified by Mr. Gowdey.

Glossinæ at night, when they are just as active as by day. They do not seem able to penetrate the smooth hard shell of a Glossina pupa; although a cracked one is soon cut up piecemeal.

When once a single ant has fastened on to anything, in an incredibly short time the prey is overwhelmed by numbers, and slowly cut up and dragged into the nest.

V. *Diptera*. *Family Asilidæ*.—The predaceous flies of this family may be expected to catch Glossinæ amongst their other prey. I have taken, at Jinja and on Damba, a fairly large species of the genus *Promachus*, which is powerful enough to catch and feed upon a species of wild honey bee, and have seen it feeding upon large *Sarcophagia* flies. Although it has not been seen to catch Glossinæ, a specimen kept in captivity readily caught and devoured Glossinæ put into the cage.

VI. *Hemiptera heteroptera*. *Family Reduviidæ*.—A carnivorous bug of this family (*Reduvius albopilosus**) was very common on the herbage in the fly area at Kerinya. It stalks its prey and catches it with the forelegs much like a mantis; and one that was kept in a cage for many weeks, and was fed on nothing but Glossinæ, thrived on the diet, since it laid many eggs. But I was never fortunate enough to see one catch a Glossina, though these often settle on the same herbage.

ARACHNIDA.

I. *Araneæ*, *family Attidæ*.—Jumping spiders abounded on the low herbage and bushes at Kerinya, and one large black-and-white species was seen devouring a dragon fly which it had caught (the species of *Cacergates* mentioned). One which was kept in captivity readily caught and devoured Glossinæ, and as it is active enough to catch such an alert creature as a dragon fly, presumably it could also catch Glossinæ, which settle on the very herbage over which the spiders are constantly hunting.

Another species, probably of the family *Lycosidæ*, was found devouring a Glossina on the ground. This was, however, possibly a maimed specimen, as I had just been marking flies on that spot.

Incidentally I may say that no Glossina has ever been seen in a spider's web.

II. *Chernetidea*.—It is worth mentioning that I have never seen one of these little "False Scorpions" attached to a Glossina, although they are very commonly found attached to the common house fly.

III. *Acarina*.—Small scarlet mites are sometimes found affixed to Glossinæ; apparently any part of body or limbs suffices. Out of 9,000 flies at Jinja 15 had one or more of these mites attached. I could never see that they were in any way harmful. They may be found similarly attached to very many different kinds of insects.

* Identified by the Entomological Research Committee.

Diseases.

Fungoid.—I have kept a careful watch for any fly attacked by a fungus, such as *Empusa muscæ*, which causes so great mortality among house flies in England in late summer; but have never found a *Glossina* so affected.

Bacilli are so frequently found in the fore-gut of the fly that they must be looked upon as normal inhabitants. Frequently the fore-gut may be seen entirely filled with them. *Micro-cocci* are also to be seen, but not so often.

Protozoal.—With a view to ascertaining whether *Glossina* is subject to Myxosporidial disease, such as is known to occur amongst other insects, the excreta of 65 flies have been carefully examined under a $\frac{1}{8}$ objective. On one occasion *Trypanosoma grayi* were seen; bacteria have never been seen. Once was seen a flat, circular capsule of the same nature as those, subsequently illustrated, from the gut of the fly. (See Fig. 3, Plate 26.)

Conclusions.

1. No vertebrate has been found to attack larva, pupa, or fly.
2. The most valuable enemies are amongst Hymenoptera, members of which attack larva, pupa, and fly.
3. No evidence has been obtained of any disease affecting *Glossina*.

IV.—BREEDING GROUNDS.

I am not able to add anything of importance to our knowledge of the breeding grounds.

At Kerinya I was unable to find more than about a dozen pupæ. There was no beach there, the shore being soft moist earth, but at one place a few yards back from the water was a deposit of gravel left by the subsidence of the lake. This was mostly covered with undergrowth and dead leaves, but at one spot amongst bushes the gravel was exposed, and here by frequent searching a few pupæ were found. On *Nsazi Island*, there being a sandy beach backed by a belt of vegetation, I expected to find many pupæ, but there were not large numbers, although more than at Kerinya. The probable reason is that fine sand lying at the base of a cliff held the moisture draining therefrom; there were several little rivulets running down the beach. Such pupæ as were found were at the edge of the belt, where they would be sufficiently in shade, but near enough to the sunlight to be kept dry.

The breeding ground on *Damba Island*, whence some 2,000-3,000 pupæ are obtained monthly for the laboratory at Mpumu, is formed of small pebbles mixed with coarse sand left by subsidence of the lake some 4-5 feet above its present level, and about the same number of yards away from the present water's edge. The belt of vegetation comes to an abrupt end (as seen in Fig. 3), and pupæ are found practically only at the edge of this belt which faces S.E., and is in shade after mid-day.

Pupæ are found collected here and there in great quantity; evidently there are a few favourite spots. One of these is seen at the extreme right of Fig. 3, where it appears as a break in the line of bushes.

Fig. 4 shows another such gap in the bushes where pupæ are found collected. These bushes have leaves very like poplar; and indeed their whole appearance suggests poplar undergrowth. I have only seen them growing on the shore; they throw a good shade, and the branches ramify a little above the ground so as to allow no undergrowth. Consequently there is free air circulation above the ground, which is itself in shade, and these two factors I believe to be of must importance, as thereby the breeding grounds are kept dry without being exposed to sun.

Such conditions can also be obtained where a tree has fallen, its branches in places arching over the ground, but keeping it in shade. A tree trunk on Damba provides an admirable place for pupæ, which may be found in hundreds in the angles. In Fig. 5, the angles in the shelter of which pupæ are collected, are indicated by deep shadow.

Both this site and the one shown in Fig. 3 were also suitable places for "Ant lions" (larvæ of *Myrmeleonidæ*), whose conical pits were abundant in the sheltered shady dry spots. This is worth mentioning, as it may help to indicate where to look for pupæ.

I have found no collections of pupæ in spots that received the mid-day sun; indeed in most places the pupæ are in shade all day, or at least after 11 a.m. Whilst watching flies deposit their larvæ at favourite spots, I have never seen one do so when in the sun; if it alighted first in the sun it always moved into a shady spot before extruding the larva.

I have witnessed the birth of eight larvæ, and it appears that the middle of the day is the favourable time. The exact times were 12, 12.15, 12.30, 10.45, 12, 1, 12.15, 12.30. I have frequently spent from 9 to 1.15, with short intervals, lying on the ground watching for flies to extrude larvæ. Until about 10 one sees no pregnant flies; then they begin to appear and rest on the pebbles, and towards mid-day they come in increasing numbers; I have seen eight resting within a square foot. But very often one sees no larvæ extruded; presumably certain atmospheric conditions are required to complete the stimuli necessary.

The actual extrusion is a very quick process; after the large hinder extremity of the larva is free on the ground, the female takes a step forwards as if to draw out the rest of the larva, and then remains motionless some fifteen minutes before flying away. The larva at once sets to work to bury itself, which it does within a minute or two.

The females containing large larvæ are heavy on the wing, and one soon got to recognise the sound of their flight. While they are resting on the ground there is obviously more chance of them succumbing to an enemy than a normal fly. Small ants, of the species previously mentioned, very often came near, and if they touched the leg of a fly it would sidle off quickly to a little distance, obviously alert; and if the ants were troublesome the fly would take a short flight, often returning to the same spot.



FIG. 3.—A general view of the fly ground on Damba Island. At the gap in the bushes on the extreme right, hundreds of pupæ may be found collected.



FIG. 4.—In the gap shown in the line of bushes many pupæ are collected.



FIG. 5.—Fallen tree trunk, under the shelter of which, in the angles appearing as dark shadows, many pupæ may be found.

As regards hatching out of the flies, I notice that meteorological conditions determine this to a great extent; fine dry weather causes many more to emerge than wet weather.

Conclusions.

1. Favourite sites for depositing pupæ are those which are in shade, but where there is free air circulation.
2. The soil, commonly gravel or coarse sand, must be dry and loose.
3. The fly does not extrude its larva until the middle of the day, always selecting a shady spot.

V.—FOOD SUPPLY.

It has for long been known that the proportions of the sexes of *Glossina* differ extremely on mainland and islands.

It has been thought that this is accounted for by the supposition that females thrive best on mammalian blood, of which there is a more plentiful supply on the mainland, and that on islands where non-mammalian blood is the more readily obtained the males thrive best and are therefore more numerous.

Mammalian and non-mammalian blood will be henceforth mentioned as M. and N.

To ascertain whether the supposed preference of females for M. is a valid explanation of the sex inequality, the gut-contents of flies were examined, the sex of each fly being noted.

At Kerinya, on the mainland, where the sex proportion was found, on examination of over 12,000 flies to be

$$\text{♂} : \text{♀} :: 47 : 53$$

300 flies of each sex were examined microscopically, and of these

$$\text{♂} 59 \text{ and } \text{♀} 33$$

contained blood whose source was recognisable. In these 92 cases the proportions were

$$\text{M} : \text{N} :: 31.5 : 68.5.$$

In the 50 ♂ the proportions were

$$\text{M} : \text{N} :: 27.1 : 72.9,$$

and in the 33 ♀ the proportions were

$$\text{M} : \text{N} :: 39.4 : 60.6.$$

It is of interest to note that the blood ingested in three cases contained *Hæmogregarines*, and in two cases *Halteridia*. In one instance both were seen in the same blood, the sharply hooked tip of the *Hæmogregarines* which were unpigmented, and the pigment granules in the *Halteridia*, being plainly discerned. This suggests that a bird had been fed upon.

Again, on Damba Island, where examination of over 6,000 flies showed the sex proportions to be

$$\text{♂} : \text{♀} :: 78.4 : 21.6$$

455 flies were examined and of these the nature of the blood was recognisable in

♂ 79 and ♀ 35.

In these 114 cases the proportions were

M : N :: 21 : 79.

In the 79 males the proportions were

M : N :: 21·5 : 78·5,

and in the 35 females the proportions were

M : N :: 20 : 80.

From these figures, more especially those compiled from Damba statistics, it will be seen that the proportions in each sex of M and N are not sufficiently different from the proportions of M and N in the whole number of flies to justify the assumption of female preference for mammalian blood. Moreover, the percentage of N is invariably much greater, though this difference is shown most obviously on the islands.

Sources of Blood.

At Kerinya the sources of mammalian blood were monkeys, a species of mongoose, bats, and other small insectivorous animals, rodents, hippopotami, wild swine, bushbuck and waterbuck.

Crocodiles were seldom seen on Kerinya, but monitor lizards (*Varanus*, locally known as Nswa-swa) were abundant.

On Damba Island the mammals are fewer in species; monkeys, bats, shrews, rodents, hippopotami, and tragelaph (or marsh buck, locally known as *sitatunga*) being all that I have noted. As regards bats it is worth mentioning that they may be found by day hanging amongst shady vegetation, and that *Glossinae* are constantly found in the same locality; and that a bat would offer an easy supply of food.

The source from which non-mammalian blood chiefly comes, on Damba Island, is, I think, either the cormorants and darters, or *Varanus*. About 100 yards away from the shore of the peninsula on which is the camp are rocks appearing out of the water, on which invariably these birds sit, and *Varanus* also often suns itself there. I have seen two on one rock, and it is often met with on the shore.

Varanus.

In order to see whether *Glossina* visits these rocks I landed from a canoe, and two at once appeared, of which there had been no signs in the canoe.

I consider *Varanus* to be by far the most important reptilian food supply; crocodiles are scarce, and I have only once seen a small one in the neighbourhood, whereas *Varanus* is very common. On one occasion, when I was watching for flies depositing larvæ, a half-grown *Varanus* crawled out from some bushes and lay in the sun within a few feet of me. Several *Glossinae* at once pounced upon him, in spite of the fact that I, being a mammal, was presumably a more enticing source of food, and I watched one fly seated on the tip of his nose, slowly swell out with blood until it flew away. The *Varanus* soon moved off slowly along the

beach, and I, following, was able to see a little swarm of *Glossinae* accompanying him, often sitting on his back and being brushed off by either fore or hind legs. On another occasion a full-grown *Varanus* came close to me, only the stems of a bush intervening; and although I could not see the whole of him, yet the frequent movements of his legs showed that he was constantly brushing flies off his back.

Water.

As regards the question whether *Glossina* drinks water, I have watched carefully, but never saw it do so. At Kerinya, where the lake shore was muddy, and hippo tracks formed little pools, many bees and syrphid flies used to come and drink, but *Glossinae* never. Indeed I rarely saw them settle at all on mud. Also, in early morning, when leaves were still wet with dew, I have seen *Glossinae* settle on them, but never once did a fly suck up a dew drop.

Vegetable.

As regards vegetable juices, never have I seen a *Glossina* with its proboscis inserted into vegetable tissues, though it must be admitted that at Kerinya there was nothing of the nature of juicy fruits within the fly area. On Damba Island 455 flies have been examined, special attention being directed to the vegetable organisms to be found.

The method adopted was to snip off the extreme tip of the abdomen, gently press out the viscera, separate the gut, and compress it under a cover slip.

To prevent the preparation drying a drop of fluid was necessary. Now, citrate and saline solutions, such as are used for trypanosome preparations, are open to the objection that they may introduce organisms into the preparation. Pure rain water was therefore used, which had been collected into a clean bottle from the tent roof after it had been continuously washed for a couple of hours by a tropical deluge.

Even with this precaution, however, organisms were introduced into the preparations. A form of alga (see Fig. 1 of Plate 26) extremely often found was once seen in a blood film from a monkey. It was eventually found to have come from the silk rag used for wiping coverslips, and must have been put on to the coverslip when polishing it before making the blood preparation.

Control preparations were therefore made at frequent intervals by moistening the rag with a little of the rain water used, and squeezing out a drop, which was then carefully examined.

Any organisms thus found were noted and discarded from the list of organisms found in fly preparations. The following organisms, of which drawings have been made, may therefore be taken to have genuinely come from the gut of the fly.

Fig. 2.—

From a female fly, Damba 62.

A small piece of a linear alga. It was doubled on itself near one extremity. The cells are of somewhat ovoid shape, and the protoplasm of one bulged into the lumen. This alga has only been seen once.

Fig. 3.—

From male fly, *Damba* 78.

In the preparation was a mass of *débris*, in which were the objects figured. 1 and 2 appear to be flat circular capsules in which no structure could be made out; one was ruptured, probably by pressure from the cover glass in making the preparation. The dark rim was due to the upper and lower surfaces being in contact at the periphery. About a dozen of these were found in the preparation. The object referred to previously (p. 95) as having been found in the excreta was of this nature. Pieces of alga were also found (3, 4).

Fig. 4.—

Male fly, *Damba* 110.

The objects figured consisted each of eight pairs of bluntly pointed cells, joined at their bases, enclosed in a very thin common membrane. Each of these cells contained dark granular protoplasm, and a varying number of oil globules of various sizes. The colour was greenish. Nineteen of these groups were seen, but in some the membrane had ruptured so that the contained bodies were scattered, but still joined in pairs. These have not been seen in any other fly.

Fig. 5, female fly, *Damba* 116; Fig. 6, female fly, *Damba* 166; Fig. 7, male fly, *Damba* 202 (Fig. 7 is on Plate 27).

I give these drawings with some hesitation. The objects are very peculiar, with thick hyaline walls. Cell spaces appear at the growing end where the walls are thinnest, and become difficult to make out towards the other end, at which are thick dark septa, one of which forms the extremity.

In Fig. 7 oil globules are present at the growing end; in the others were only granular cell contents. These organisms are very often found, and sometimes 12-16 may be seen in one preparation.

On examining control preparations from the silk rag, one or two were occasionally found therein; but taking into account the relative scarcity in control preparations, and the numbers found in one fly, I am disposed to think that those obtained in the controls had originally got on to the rag when a coverslip from a fly preparation in which there had been many had been wiped dry after being put in water.

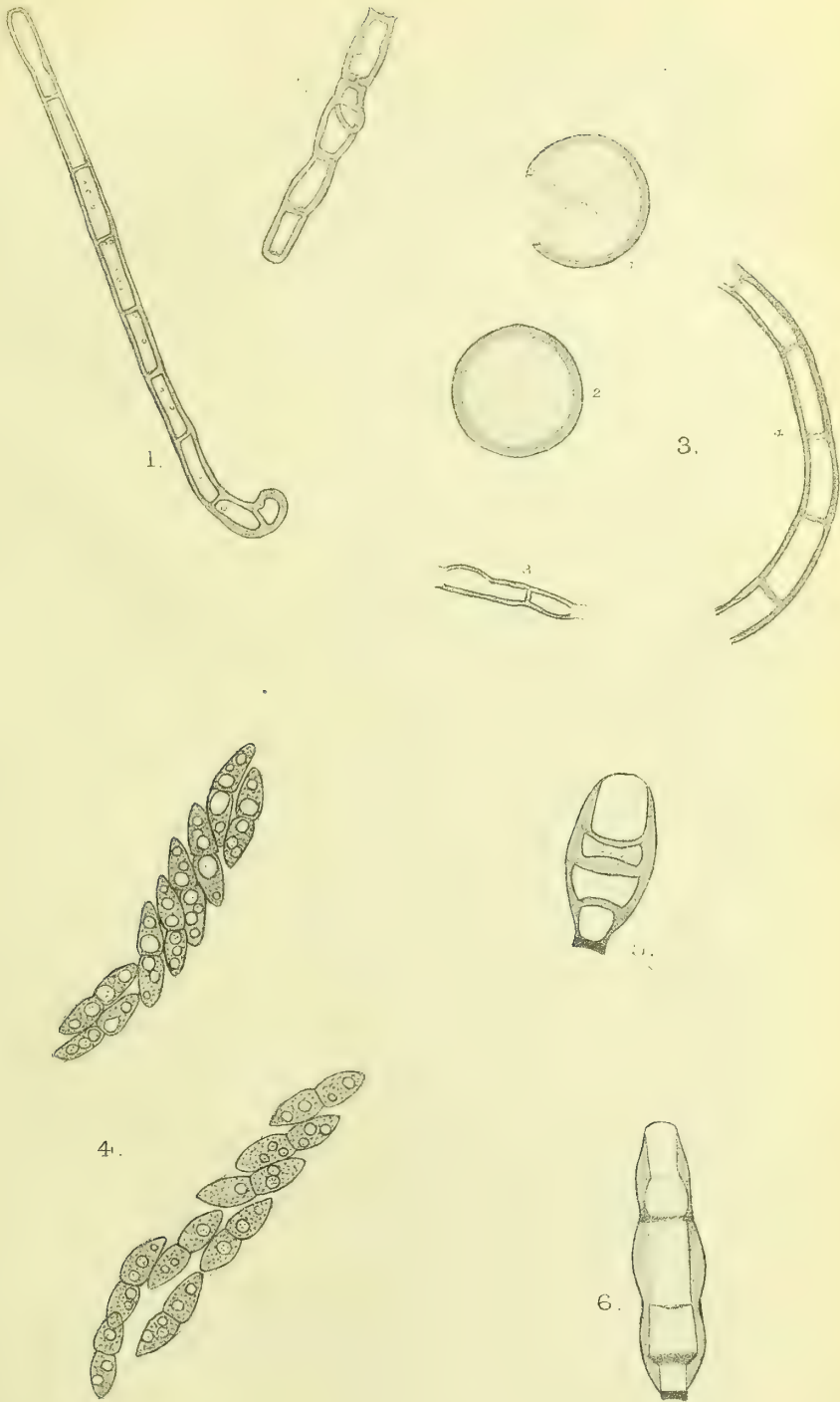
In support of this it may be said that those found in the controls usually presented an altered or broken appearance.

These organisms were frequently seen in preparations from flies made at Jinja. It is possible, therefore, that they really do come from the gut.

Fig. 8.—(Plate 27.)

Male fly, *Damba* 133.

A large, thick-walled vegetable cell, broken off from its neighbour, from which it is separated by a septum strikingly like a sieve-plate. It contained granular protoplasm irregularly distributed.



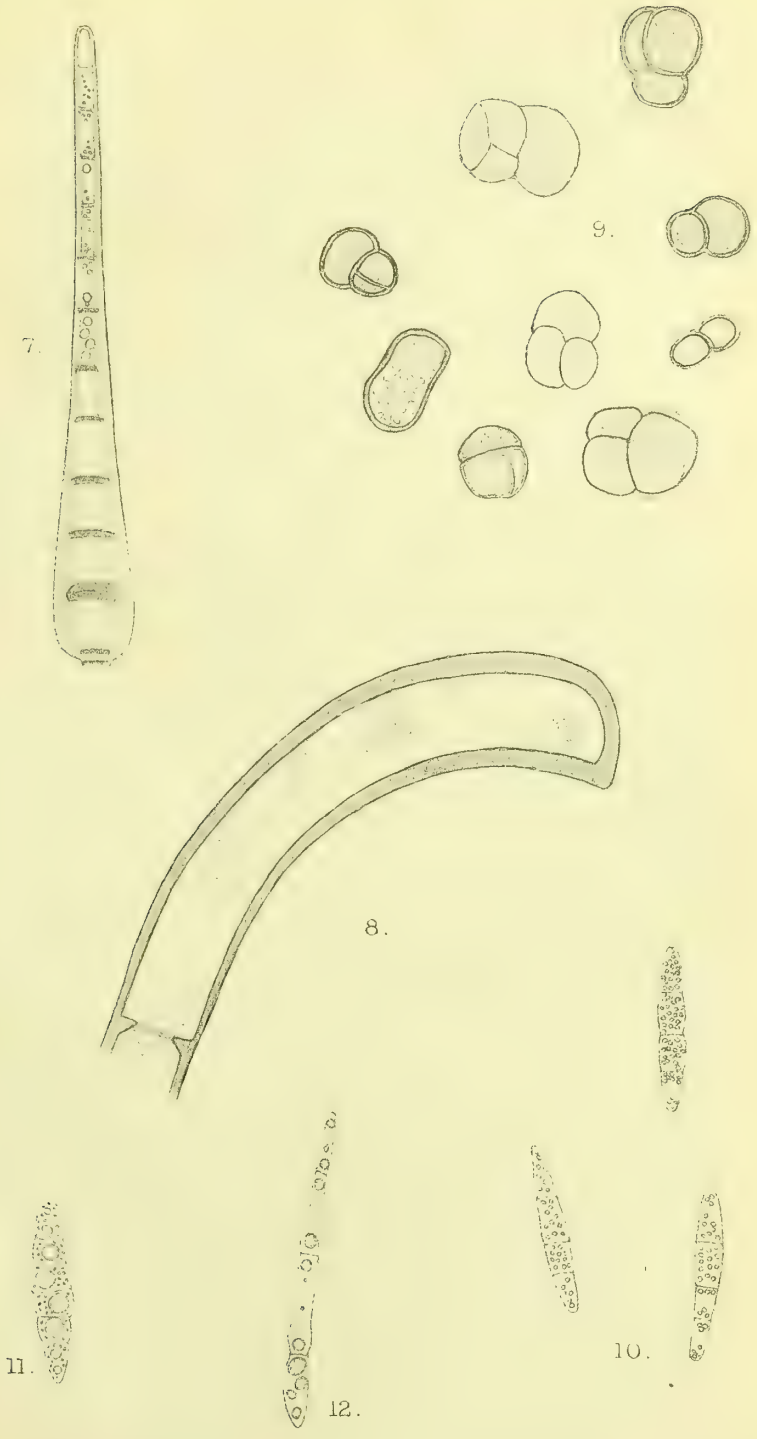


Fig. 9.—

Female fly, Damba 118.

A large number of poly-cellular objects, containing granules but no oil globules, with walls of varying thickness, they were of a faint green colour and rather opaque.

Fig. 10.—

Male fly, Damba 145.

These quadricellular, very pale, transparent bodies, containing oil globules were seen in one fly only, in which they occurred in great numbers. They were very faintly green.

Fig. 11.—

Female fly, Damba 167.

A similar body, of six cells, more swollen in the middle, and quite colourless save for a green tint in the walls, contains large oil globules, and refractile granules. Has only been met with once.

Fig. 12.—

Female fly, Damba 188.

Somewhat like Fig. 19, but tricellular, longer and thinner; the septa were very indistinct. Only one has been seen.

Fig. 13 (Plate 28).—

1. From ♀ fly, Damba 264.

2. From ♂ fly, Damba 367.

Comparatively large bodies of very curious nature, not met with frequently; they were also seen at Jinja.

There is a translucent ground substance in which are cells containing barely discernible small granules. At one end is a dark, opaque body, situated in one specimen at the end of a granular area.

Fig. 14.—

Female fly, Damba 298.

The drawing is a focal section of the body, which had more than one plane of cells. The whole was rather opaque and greenish. Each cell contained a large nucleus, but no oil globules.

Fig. 15.—

1, 2. Female fly, Damba 299.

3, 4, 5. Male fly, Damba 367.

In these flies very curious bodies were seen. There was a homogeneous central substance enclosed in a very thick wall of highly refractile substance, which was perforated by numerous canals, and through these projected processes from the central mass. No movement was ever observed.

1 is a focal section showing canals and a process passing through one.

2 is a surface view of the same body, which was roughly spherical, and the projecting processes were seen on the surface as refractile points.

3, 4, 5 were from another fly, they were larger and polygonal. These are outlined only.

In a third fly four of these bodies were seen; they were narrow rectangles in shape, and two were apposed end to end.

Fig. 16.—

Female fly, Damba 335.

This large, three-celled structure contained abundant granular protoplasm but no oil globules. It has only been seen once.

Fig. 17.—

Female fly, Damba 440.

Apparently a ruptured capsule of spherical shape; affixed all over the surface were granules, some of which were detached in the preparation and were undergoing vigorous Brownian movement. The body was only seen once; it rather resembled a minute fungus *sporangium*.

Fig. 18.—

Female fly, Damba 454.

In the proctodæum of one fly were found two bodies, each composed of quadrants which in one case seemed on the point of separating; in the other each contained a spherical nucleus of paler appearance than the rest of the homogeneous cell contents.

From these drawings it appears that various organisms are found in the fly's gut which have certainly in some cases multiplied therein very freely, as evidenced by the numbers found in one fly only. They may possibly be true symbionts; it is significant that none were seen containing chlorophyll.

Conclusions.

1. The varying proportions of the sexes in different localities are not accounted for by the nature of the blood food supply.

2. Flies have not been seen to drink water or feed on vegetable juices.

3. Numerous vegetable organisms have been found in the gut of *Glossina*, whose origin is not known. Possibly they are symbionts.

VI. BACILLI AND TRYPANOSOMES, &c.

During the examination of the 600 flies at Jinja for the nature of the food, the frequency of occurrence of bacilli and trypanosomes was noted.

Occasionally, on removal of the gut, the fore-part of it was seen to be of a bright carmine colour, and microscopically this was found to be due to a mass of amorphous granules of a translucent carmine colour, lying in the lumen of the gut. These will be alluded to as carmine granules.

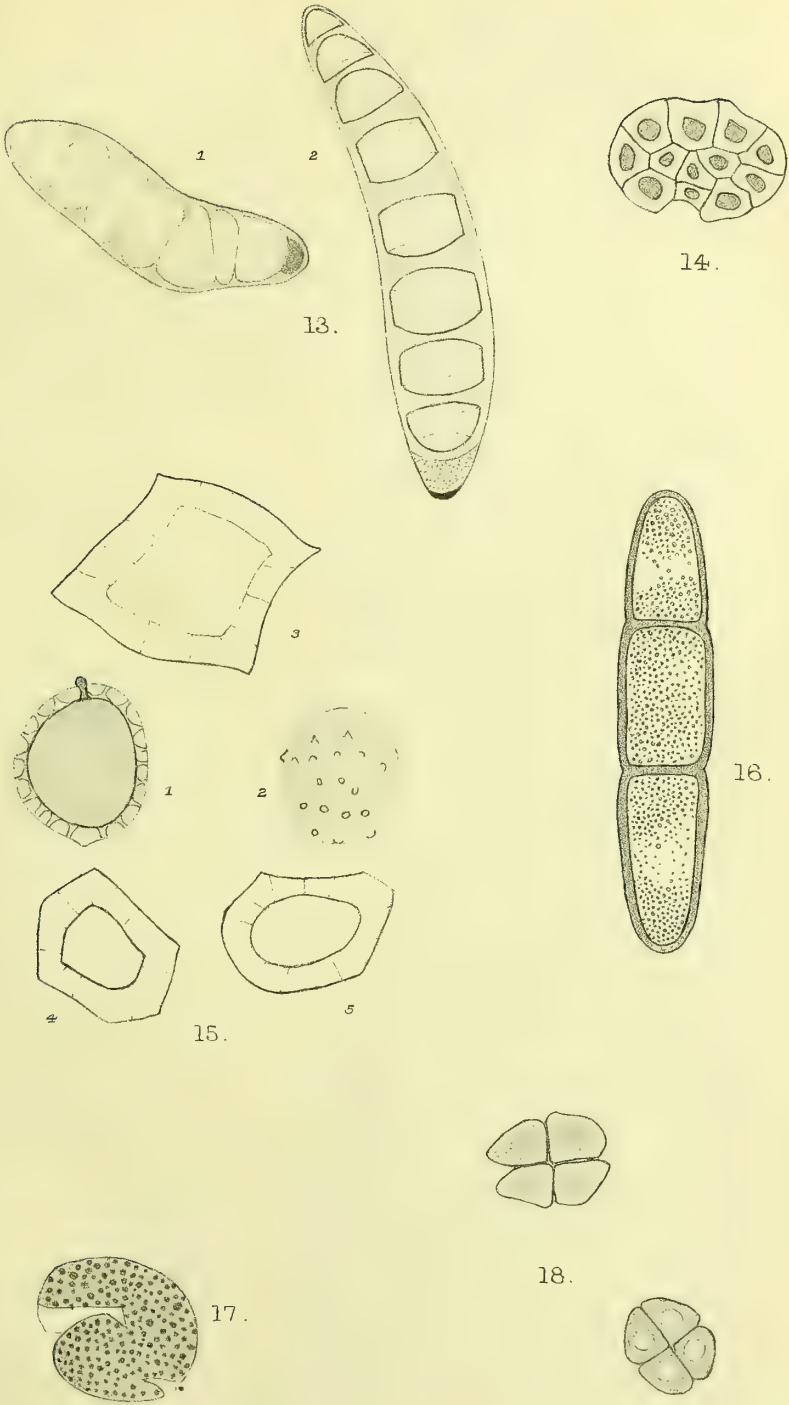


Table V.

—	T. Trypanosomes.	B. Bacilli.	G. Granules.
1. Percentage of total flies containing either T. B. or G.	11	19·3	5·5
2. Percentage of each heading, of males.	43·9	50·8	33·3
3. Percentage of each heading, of females.	56·1	49·2	66·7
4. Percentage of cases under each heading in which T. also occurred.	—	3·4	51·5
5. Percentage of cases in which B. also occurred.	6·1	—	9·1
6. Percentage of cases in which G. also occurred.	30·3	2·6	—

From Table V. it will be seen that there is not much difference between the sexes as regards frequency of occurrence of bacilli or trypanosomes, and that it is extremely uncommon to find both in the same fly. In those cases where both did occur one or other was usually in the minimal quantity.

. In only 3·4 per cent. of the flies containing bacilli were trypanosomes also found, and in only 6·1 per cent. of flies containing trypanosomes were bacilli found.

As regards the carmine granules, which were found in 5·5 per cent. of all the flies, there would seem to be some connection between them and trypanosomes.

Very often when the gut was found to be pink, on removal, it was shown to be swarming with myriads of trypanosomes. In 51·5 per cent. of the cases wherein these granules were found there were also trypanosomes, and in 30·3 per cent. of the cases where trypanosomes were present the granules were also seen. But of the cases in which bacilli were present only in 2·6 per cent. were the granules also found, and of the cases of granules only 9·1 per cent also contained bacilli.

Thus whilst there is no connection between bacilli and the granules, they are in some way favourable to the multiplication of trypanosomes. Of the nature of these granules I can say nothing, save that they are non-crystalline and insoluble in alcohol or water. They are twice as numerous in female as in male flies.

Conclusion.

There is marked incompatibility between trypanosomes and bacilli in the gut of the fly.

VII. THE CONTENTS OF THE GUT OF FRESHLY-BRED FLIES.

From the marked incompatibility between bacilli and trypanosomes in the fly's gut, it seems possible that the former may inhibit the multiplication of the latter, or that the conditions suitable for one are not so for the other. If one could infect recently-bred flies with a culture of the bacillus it might be possible by subsequently feeding the flies on an animal whose blood

contained trypanosomes, and then on a normal animal to determine whether the bacilli had inhibited the development of the trypanosomes—*provided that* the bred flies contained no bacilli. Accordingly a number of freshly-hatched flies have been examined, only those being chosen which have just emerged from the pupa, being still soft, with proboscis bent under the thorax. No fluid was necessary to moisten the preparation, as the fly's abdomen was full of fluid; the contents were simply gently pressed out after snipping off the top of the abdomen. 205 flies have been examined, and bacilli were present in 9·8 per cent.

In addition, one often found the large crystals commonly seen in the gut of wild flies; these are square or rectangular, sometimes resembling calcium oxalate crystals, sometimes the "coffin" crystals of triple phosphate.

Of much interest is the fact that various organisms have been found in the gut of these bred flies.

Figs. 19, 20, 21 (Plate 29).—

Show organisms of the same nature as Figs. 14, 15, 16.

Fig. 22.—

A similar body but considerably darker with more opaque walls.

Fig. 23.—

This body was found in a female fly, and another one in a *larva*.

It is rather faint and indistinct, and with thick, very clear walls, and contains oil globules.

Fig. 24.—

Possibly a young specimen of that shown in Fig. 25.

Fig. 25.—

Of same nature as the bodies shown in Fig. 18.

Organisms found in Bred Flies.

Fig. 26.—

This organism had the appearance of a minute larval nematode. The cuticle was of shining yellow refractile material, but quite smooth everywhere. The body was very granular, save at the extreme ends; it was immobile and coiled up. This is of much interest.

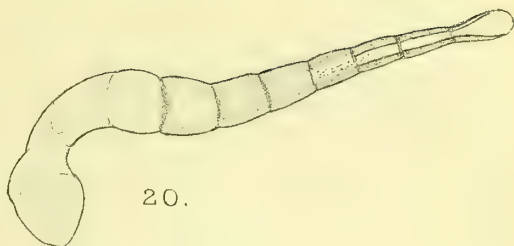
I have not personally met with a nematode in any wild fly examined, but Capt. Gray found an immature female of a species of *Mermis* in the body-cavity of *Glossina*.

Conclusion.

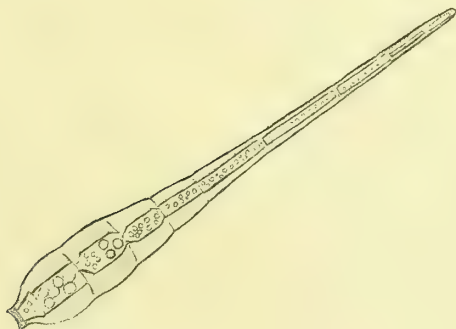
1. Bacilli are present in the gut of 9·8 per cent. of bred flies.
2. Various other vegetable organisms are also hereditarily transmitted.
3. An organism believed to be a larval nematode has been found in a bred fly.



19.



20.



21.



22.



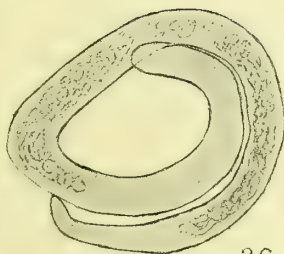
23.



24.



25.



26.

VIII.—THE SEX PROPORTIONS.

It has been shown earlier in the Report that the disproportion in sex of the fly at different localities is not explicable on the grounds of food supply. Moreover, it is not, as might be expected, due to the sexes being produced in different numbers.

Thus, of 1,400 flies bred from pupæ obtained on Damba Island, the proportions were

$$\text{♂} : \text{♀} :: 48 : 52,$$

whereas of nearly 5,000 flies caught during the period in which the pupæ were collected the proportions were

$$\text{♂} : \text{♀} :: 78.6 : 21.4.$$

This is a very striking difference.

It is worth noting that the proportions of sex in the flies *caught* at Jinja, on the mainland, were practically the same as in the flies *bred* on Damba Island—

$$\text{♂} : \text{♀} :: 47 : 53.$$

I can see nothing as yet to account for this, for it is difficult to think of any cause that would not be equally effective on the mainland.

For if the small numbers of females caught on the island were due to their death during larvi-position, or from premature pupation of the larva in utero, both of which are common in captivity, these causes would affect them equally on mainland. The obvious supposition that female flies avoid being caught is of no avail for the same reason; likewise the supposition that females do not live as long as males, apart from the fact that marking experiments do not support this.

Possibly there is, on the island, some enemy which by preference attacks the females, which when pregnant are larger, and heavier on the wing.

Conclusion.

The preponderance of males over females on the islands is not due to their production in greater numbers; the sexes are produced in very nearly equal numbers.

IX.—TSETSE-FUGES.

I have tried four substances as Tsetse-fuges, and found none of any use. Not only do they not prevent the flies biting, but they do not even prevent them settling.

I.—*Naphthalene*, 7 per cent. dissolved in olive oil supplied by the Principal Medical Officer. On one occasion, within the first half hour after liberally anointing one hand, 8 flies settled to feed on the hand, and others to rest.

II.—*Oil of White Birch*, supplied by the Principal Medical Officer. This smells strongly, like Russian leather, and is not unpleasant to use.

III.—*Essential oil of Carapa grandiflora*, supplied by the Director of the Sleeping Sickness Bureau. This has no value as a Tsetse-fuge, and is of little use in keeping off mosquitoes, unlike oil of birch.

IV.—*Infusion of Carapa grandiflora*, supplied by the Director of the Sleeping Sickness Bureau. Has a penetrating odour suggestive of formic aldehyde. On one occasion I thoroughly rubbed one hand and kept the other as a control, sitting down and placing both hands near together. In half an hour 9 flies settled to feed on the treated hand, 11 on the control. As soon as they made preparations for feeding they were brushed off. This count did not include those that merely settled and flew off again.

Sometimes the fly showed that it disliked the substance by feeling about with the proboscis to discover an untreated spot, but the dislike was not often strong enough to make it give up feeding.

Conclusion.

The four preparations tried were of no value as Tsetse-fuges.

X.—THE VITALITY OF PUPÆ.

Experiments are in progress to test the resistance of pupæ to various conditions. It has been found that they are not necessarily killed by any of the following conditions; in all cases a proportion have developed into adult flies.

In all cases part of the batch of pupæ was used as a control.

Full details of the experiments will follow in a later report. (See Appendix, p. 108.)

Effect of moisture.

- | | | |
|-------|---|------|
| I. | Pupæ were kept in a closed box with blotting-paper always saturated with water. | |
| II. | They have been totally immersed for 48 hours. | |
| III. | „ „ „ „ „ „ | 60 „ |
| IV. | „ „ „ „ „ „ | 72 „ |
| V. | „ „ „ floated on surface „ | 96 „ |
| VI. | Immersed daily for $\frac{1}{2}$ hour for 20 days. | |
| VII. | „ „ „ 1 „ „ | 7 „ |
| VIII. | „ „ „ 4 „ „ | 5 „ |

Conclusion.

Pupæ can tolerate immersion for 72 hours and flotation for 96 hours.

Effect of Sun.

Pupæ were placed in a small wooden box covered with $\frac{1}{2}$ inch to 1 inch loose earth, and the box was sunk in the ground, so that the level of the surface of its contained earth was the same as the surrounding level.

On one occasion a thermometer placed beside the pupæ registered 101° F. (maximum).

IX. Exposed to sun in middle of day daily for 1 hour for 12 exposures.

X. Exposed to sun in middle of day daily for 2 hours for 18 exposures.

XI. Exposed once to sun for 4 hours.

The much greater tolerance of pupæ than flies to sun is noteworthy.

The tolerance of immersion is noteworthy, for I have seen part of a beach where pupæ were buried washed by waves, owing to a high level of the lake and a driving wind.

Summary of Conclusion.

- I.—(A.) A female fly has been shown to live a minimum time of 182 days; a male 149 days.
(B.) A spell of dry weather is fatal to flies which have been living several months.
(C.) The decrease in relative humidity of the atmosphere is responsible for the decrease in number of flies noticed at the onset of dry seasons.
- II.—A year and ten months after removal of last inhabitant of Damba Island 885 flies caused infection of monkey.
- III.—(A.) No vertebrate has been found to attack *Glossina* at any stage.
(B.) The most valuable enemies are amongst Hymenoptera, which attack larva, pupa, and fly.
(C.) No evidence has been obtained of any disease affecting *Glossina*.
- IV.—(A.) Favourite sites for depositing larvæ are in shade, where there is free air circulation.
(B.) The soil, commonly gravel or coarse sand, must be dry and loose.
(C.) The fly does not extrude the larva until the middle of the day, always selecting a shady spot.
- V.—(A.) The varying proportions of sex in different localities are not accounted for by nature of blood food supply.
(B.) No fly has been seen to drink water, or suck plant juices.
(C.) Numerous vegetable organisms have been found in the gut of *Glossina* whose origin is not known. Possibly they are symbionts.
- VI.—There is marked incompatibility between trypanosomes and bacilli in the gut of the fly.
- VII.—(A.) Bacilli are present in 9·8 per cent. of bred flies.
(B.) Various other vegetable organisms are also hereditarily transmitted.
(C.) An organism believed to be a larval nematode has been found in a bred fly.

VIII.—The preponderance of males over females on Damba Island is not due to their production in greater numbers; the sexes are produced in very nearly equal numbers.

IX.—Four preparations tried as Tsetse-fuges were of no avail.

X.—Pupæ can tolerate total immersion for 72 hours, flotation for 96 hours, and 18 daily exposures to mid-day sun of 4 hours each.

APPENDIX.—MARCH, 1912.

EXPERIMENTS TO TEST VITALITY OF *GLOSSINA PALPALIS* PUPÆ.

The influence of varying conditions upon the vitality of pupæ of *Glossina palpalis* has not hitherto been investigated on a large scale, and the following experiments have been made to test their vitality when submersed in water, and exposed to sun. According to my experience pupæ are only found in the driest possible situations, though always close to the water's edge; so that it would appear that contact with water is inimical to them. It will be seen, however, that they have considerable capacity to resist the effects of submersion; moreover, they do not become mouldy (so long as they are alive) when kept in an atmosphere with the maximum degree of humidity. Their powers of resistance to sun are less in proportion.

The experiments were carried out thus—

A batch of pupæ, obtained from one locality at the same time, was divided into portions, one of which was reserved as a control whilst others were exposed to various conditions.

The control pupæ were kept in the hut on the Lake shore of Damba Island, where the experiments were carried out; they were in wooden boxes with glass lids, but not covered with earth. After the other pupæ had been exposed to the conditions whose influence it was desired to test they were kept under exactly the same conditions as the controls.

Effect of humidity.

Table I.

Treatment.	Experimental pupæ.		Control pupæ.	
	No. used.	Percentage of flies obtained.	No. used.	Percentage of flies.
A. Piece of blotting-paper in box sprinkled with water daily.	100	87	100	89
B. Blotting-paper always saturated.	75	85·3	75	56

Remarks.—In experiment B. it appears that the experimental conditions were more favourable than the control: nearly half

of the control pupæ dried up; though in A. the controls kept under similar conditions, mostly developed into flies.

Effect of discontinuous submersion.

Table II.

Treatment.	Experimental pupæ.		Control pupæ.	
	No. used.	Percentage of flies obtained.	No. used.	Percentage of flies.
Daily submersion for—				
A. $\frac{1}{2}$ -hour for 16 days	19	21	50	82
B. 1 " 17 "	60	73·3	110	84·5
C. 4 hours for 5 "	110	73·6	110	91·8
D. 6 " 7 "	97	82·5	100	85
E. 12 " 5 "	100	58	100	75
F. 12 " 10 "	100	48	100	89
G. 12 " 12 "	100	46	100	82
H. 24 hours with intervals of 12 hours, for four times.	100	49	100	82

In all cases during intervals between treatment and afterwards, the pupæ were kept in same conditions as the controls.

Remarks.—These experiments show that should any breeding ground be flooded at intervals by heavy rains, the conditions would not completely destroy all the pupæ. Even four successive submersions for 24 hours only destroyed half the number.

Experiment A. seems to show that it is rather the frequency of submersion than the total time that is adverse to the pupæ.

Effect of continuous submersion.

Table III.

No. of hours submersed.	Experimental pupæ.		Control pupæ.	
	No. used.	Percentage of flies obtained.	No. used.	Percentage of flies obtained.
A. 48	25	52·	50	82·
B. 60	44	38·7	43	65·1
C. 72	50	28·	50	64·
D. 84	110	38·2	110	91·8
E. 96	110	14·5	110	84·5
F. 108	100	2·	100	85·
G. 120	100	0·	100	82·

Remarks.—These experiments show that complete submersion of between 108-120 hours is necessary to totally destroy pupæ; and that the number that survive is in inverse ratio to the length of submersion.

Effects of flotation.

Table IV.

No. of days floated.	Experimental pupæ.		Control pupæ.	
	No. used.	Percentage of flies.	No. used.	Percentage of flies.
A. 4	110	47·3	110	84·5
B. 8	100	19·	100	85·
C. 10	100	0·	100	89·
D. 14	100	0·	100	75·

Remarks.—A certain proportion can survive flotation for eight days, but none for 10 days. Pupæ which sink are nearly always dead—if, of a batch, those that sink are set aside scarcely one will produce a fly. The power of floating is quite independent of any air bubble enclosed between the posterior processes; if this be brushed away a living pupa will still float.

Effect of submersion followed by exposure to sun.

Table IV.

Treatment.	Experimental pupæ.		Control pupæ.	
	No. used.	Percentage of flies.	No. used.	Percentage of flies.
A. 4 hours submersion one day, exposure to sun for 2 hours next day.	93	74·8	100	85
B. 12 hours submersion one night, 4 hours sun next day.	100	1·	100	75·

When exposed to sun the pupæ were put in a small wooden box, then covered with earth to the depth of one inch, and the box sunk flush with the surrounding surface, during the hottest part of the day.

Remarks.—Though neither 12 hours submersion nor 4 hours exposure to sun have, alone, much effect upon pupæ, when employed alternately they are fatal.

Effect of a single exposure to sun.

Table V.

Treatment.	Experimental pupæ.		Control pupæ.	
	No. used.	Percentage of flies.	No. used.	Percentage of flies.
A. 4 hours exposure to sun (<i>covered</i>).	110	81·8	110	84·5
B. 6 hours exposed to sun (<i>covered</i>).	100	88·	100	85·
C. 3 hours, <i>uncovered</i>	100	0·	100	89·
D. 1 hour ,,	100	0·	100	82·

Remarks.—Six hours exposure to sun during the hottest part of the day does not harm pupæ covered with one inch of earth. When uncovered they are all killed by a single hour's exposure to sun.

Effect of repeated exposures to sun.

Table VI.

Treatment.	Experimental pupæ.		Control pupæ.	
	No. used.	Percentage of flies.	No. used.	Percentage of flies.
A. Sun 2 hours daily for three days.	98	80·5	100	85·
B. Sun 3 hours daily for three days.	100	2·	100	82·
C. Sun 6 hours daily for two days.	100	0·	100	75·
D. Sun 4 hours daily for four days.	110	0·	110	91·8

In these four experiments the pupæ were covered with 1 inch of earth when exposed to the sun during the middle of the day.

13.—THE TRANSMISSION OF *TRYPANOSOMA NANUM* (LAVERAN).*

By Dr. H. L. DUKE.

On March 17, 1911, Dr. van Someren kindly sent a goat to Mpumu, which he had inoculated with a trypanosome found by

* Reprinted from the Proceedings of the Royal Society, Ser. B, vol. 85, 1912.

him in cattle in the neighbourhood of Sebwe River. This trypanosome, he averred, caused a fatal disease. On further investigation, it proved to be identical with *Trypanosoma nanum*, both as regards morphology and animal reactions, and thus confirms the opinion of the 1908-10 Commission, namely, that this trypanosome is an Uganda species.

Although experiments are still in progress, sufficiently important results have already been obtained to warrant their being recorded, adding, as they do, one more to the long list of trypanosomes carried by *Glossina palpalis*.

MORPHOLOGY.

A. *Living, Unstained.*

Corresponds closely to *Trypanosoma pecorum*. The tendency to stick to adjacent corpuscles is very marked.

B. *Fixed and Stained (Osmic and Giemsa).*

As regards general structure, agrees with the description given by former workers in this laboratory. No trace of a free flagellum is visible.

Few observations are available as regards length, owing to the limited number of susceptible animals inoculated. The following table gives maximum, minimum, and average measurements of the trypanosome in ox and sheep.

Experiment No.	Animal.	Fixation.	Stain.	In microns.		
				Maximum.	Minimum.	Average.
59	Sheep	Osmic and abs. alcohol.	Giemsa	16	12·00	14·5
232	Ox	"	"	15·50	11·00	13·5

ANIMAL REACTIONS.

The original goat (688) sent by Dr. van Someren shortly after inoculation is at present (October 20, 1911) apparently in excellent health. Trypanosomes are visible in its blood only at rare intervals.

The following animals were sub-inoculated from goat, Experiment 688:—

Date.	Animal.	Incubation, in days.	Duration of disease.	Remarks.
17.3.11	Monkey 689	—	—	No trypanosomes seen for 54 days
27.3.11	" 712	—	—	" " 54 "
15.4.11	" 7	—	—	" " 38 "
17.3.11	White rat 690	—	—	" " 63 "
27.3.11	" 713	—	—	" " 50 "
19.4.11	" 12	—	—	" " 34 "
19.4.11	Puppy 11	—	—	" " 49 "
26.5.11	" 89	—	—	" " 60 "
17.5.11	Wild pig	—	—	" " 60 "
16.5.11	Sheep 59	10	—	In good health after 154 days.
17.5.11	Goat 64	6	64	Died of <i>T. nanum</i> .

Transmission of Trypanosoma nanum by Glossina palpalis.

In the following experiments, laboratory-bred flies were employed, hatched from pupæ brought from Damba Island. The first six attempts at transmission failed, and the brief summary of these experiments given in Table I. will suffice.

It will be noticed that in every case the flies were first fed upon Goat 688, the animal received from Toro.

Table I.

Experiment No.	Date of commencement.	Number of days.		Number of flies.		Result.	Animal on which flies were fed.
		On Goat 688.	Experiment lasted.	At 30th day.	Dissected during experiment.		
25	27.4.11	10	50	16	17	—	Normal calf 43.
27	29.4.11	8	48	19	22	—	"
32	1.5.11	6	49	12	16	—	"
36	3.5.11	4	47	28	28	—	"
57	15.5.11	3	56	35	47	—	"
86	25.5.11	7	47	30	43	—	"

A further set of experiments were now started and Sheep 59 employed for the first time for the infecting feeds. It was found that the flies fed much more readily upon the sheep, and, as will be seen below, a considerable number of positive flies resulted.

A brief recapitulation of each experiment is given below, the description of the positive flies being dealt with in Table II. :—

Experiment 213.

Dat.			Day of experiment.	Procedure.	Result.	Remarks.
July 1911.						
July 21-29	...	1-8		Fed on sheep 59...		<i>T. nanum</i> ++ in sheep 59 on July 19, 21, 22, 25.
" 30	...	9		Starved.		
" 31-Aug.16		10-26		Fed on normal calf 232.	—	
Aug. 17	...	27		Starved.		
" 18-Sept. 5		28-46		Fed on goat 307...	—	54 flies alive on 30th day of experiment. Remaining flies, ♂ 10 ♀ 14.
Sept. 6	...	47		Starved and dissected.		

Note.—Of the 62 flies dissected during this experiment one female was found infected (*cf.* Table II.)

Experiment 235.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1911.				
July 31—Aug. 3	1-3	Fed on sheep 59		<i>T. nanum</i> + +, in sheep 59 on Aug. 2 and 3.
Aug. 4	4	Starved.		
„ 5—Sept. 16	5-47	Fed on normal calf, 232.	+ (?)	62 flies alive on 30th day of experiment.
Sept. 17... ..	48	Starved.		
„ 18-21	49-52	Fed on dog X.	—	Dog X died 22.9.11.
„ 22-26	53-57	Fed on dog, 399.		Dog negative to trypanosomes for 27 days.
„ 27... ..	58	Starved and dissected.		Remaining flies, ♂ 2 ♀ 27.

Note.—83 flies were dissected in the course of this experiment and of these 4 females were found to contain flagellates on the 58th day of the experiment (*cf.* Table II).

Experiment 238.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1911.				
Aug. 2-3	1	Fed on goat, 688.		<i>T. nanu</i> + in goat 688 on Aug. 2.
„ 4	2	Starved.		
„ 5—Sept. 16	3-45	Fed on normal calf, 232.	+ (?)	61 flies alive on 30th day. Calf showed <i>T. nanum</i> 18.9.11.
Sept. 17... ..	46	Starved.		Dog X died 22.9.11.
„ 18-21	47-50	Fed on dog X.	—	Dog negative to trypanosomes for 27 days.
„ 22-26	51-55	Fed on dog, 399.		
„ 27... ..	56	Starved.	—	Negative to trypanosomes for 20 days.
„ 28—Oct. 3	57-64	Fed on bushbuck, 396.		Remaining flies, ♂ 16 ♀ 14.
Oct. 4-5	65-66	Starved and dissected.		

Note.—65 flies dissected during this experiment. One female found positive (*cf.* Table II).

Experiment 243.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1911. Aug. 3-5 ...	1-2	Fed on sheep 59		<i>T. nanum</i> ++ in sheep on Aug. 3 and 5.
" 6 ...	3	Starved.		
" 7—Sept. 16	4-44	Fed on normal calf 232.	+ (?)	77 flies alive on 30th day. Calf showed <i>T. nanum</i> on 18.9.11.
Sept. 17 ...	45	Starved.		
" 18-21 ...	46-49	Fed on dog X ...		Dog X died 22.9.11.
" 22-26 ...	50-54	Fed on dog 399 ...	—	Dog negative to trypanosomes for 27 days.
" 27 ...	55	Starved.		
" 28—Oct. 3	56-61	Fed on bushbuck 396.	—	Bushbuck negative to trypanosomes for 20 days.
Oct. 4-7 ...	62-65	Fed on cock.		
" 8-9 ...	66-67	Starved and dissected.		Remaining flies, ♂ 7, ♀ 13.

Note.—Total flies dissected in this experiment 112, of which 6 females and 3 males were found infected with flagellates (*cf.* Table II).

It will be seen that in the above four experiments the flies were fed upon Calf 232, which became infected about September 10 or 11. It is thus impossible to decide which experiment actually caused the infection, though some evidence on this point may be gathered from Table II.

Dissection of the Positive Flies of the above Experiments.

All positive flies were examined by Miss Robertson, and an account of the morphology of the flagellates will appear in due course in her report. I have here to express my obligation for information regarding the parts of the alimentary tract infected, with which Miss Robertson has supplied me throughout.

In the following Table II. the flies are arranged in a progressive series, according to their age when dissected, all the flies used being newly hatched at the commencement of the experiment. The “+” and “—” signs are used to indicate presence or absence of flagellates in the various regions of the alimentary canal, and the intensity of an infection is indicated by repetition of

the “ + ” sign. The symbol “ 0 ” denotes that no observations were recorded concerning that portion of the gut:—

Table II.

Date.	No. of fly.	Age of fly when dissected.	No. of experiment.	Sex.	Region of gut.					Injection into Goat 329.
					Hind gut.	Thoracic gut to junction with proventriculus.	Proventriculus.	Salivary glands.	Proboscis.	
1.8.11	1	11th day	213	♀	++	—	—	—	—	Contents of proventriculus injected into Goat 329.
17.8.11	2	14th "	243	♀	++	+++	—	—	—	
25.8.11	3	22nd "	243	♀	+++	—	—	—	—	
28.8.11	4	25th "	243	♀	+++	+++	+++	—	+++	
29.8.11	5	26th "	243	♀	+++	++	+	—	—	Proventriculus of each of these flies injected subcutaneously into Goat 329.
5.9.11	6	33rd "	243	♀	+++	+++	0	—	+++	
18.9.11	7	46th "	243	♂	+++	0	+	—	—	
20.9.11	8	48th "	243	♂	+++	0	—	—	+	
20.9.11	9	48th "	243	♀	++	—	—	—	—	
27.9.11	10	58th "	235	♀	+++	+++	+++	0	—	
27.9.11	11	58th "	235	♀	+++	+++	+++	0	—	
27.9.11	12	58th "	235	♀	+++	0	—	0	—	
27.9.11	13	58th "	235	♀	+++	++	+++	0	++	
5.10.11	14	64th "	238	♀	+++	+++	++	—	+++	
9.10.11	15	67th "	243	♂	+++	—	—	—	—	

Note.—Goat 329, under examination 56 days, has never shown trypanosomes.

From the above it would appear that the development of *Trypanosoma nanum* in *Glossina palpalis* commences in the hind gut and extends forward *viâ* the thoracic gut and proventriculus until finally the proboscis is reached. The salivary glands are apparently not invaded by this trypanosome. A fly with a negative proboscis is presumably not infective, this conclusion being supported by the fact that on three occasions injection of positive proventriculi failed to infect Goat 329.

Fly No. 4 shows that flagellates may be well established in the proboscis by the 25th day after the infecting feed, although apparently neither this fly nor No. 6 accounted for the infection of Calf 232 on September 10 to 11.

Fly No. 8 showed a few flagellates in the proboscis, while both proventriculus and thoracic gut were negative, and the proventricular infection of fly No. 14 was relatively slight in conjunction with a swarming proboscis. These suggest the possibility that infection of the proventriculus may be merely a temporary invasion, while the flagellates are becoming established in the

proboscis, and not a permanent station, in which case the condition of Fly 8 is intelligible.

As regards the actual infecting fly in the above experiments the choice rests between Nos. 8, 13, and 14. All three flies were fed on Calf 232 from September 11 to 16, *i.e.*, during the incubation period, and may thus have derived their flagellates secondarily from this source. This applies especially to Nos. 13 and 14. There is, however, no reason why both these flies should not have become infected originally at the commencement of the experiment, in which case differential diagnosis is impossible. In the case of flies Nos. 9 and 15 there can be little doubt that the flagellates were derived from Calf 232.

As regards the position of the flagellates in the proboscis, the labrum is the chief seat of infection, enormous numbers being found in this situation. In one instance only, fly No. 4, Table II., were trypanosomes observed in small numbers in the hypopharynx.

CONCLUSIONS.

(1) That the trypanosome received from Sebwe River* is *Trypanosoma nanum*.

(2) That this trypanosome can be transmitted by *Glossina palpalis*, the proportion of positive flies obtained being relatively large, and indicating that this fly may play an important part in the spread of the disease in Uganda.

14.—ANTELOPE AND THEIR RELATION TO TRYPANOSOMIASIS.†

By Dr. H. L. DUKE.

(With Plate 30.)

The flies on the Chagwe Lake-shore are still capable of infecting monkeys with *Trypanosoma gambiense*. Four years and a half have now elapsed since the Chagwe coast line was officially free of population, all villages destroyed within a zone of two miles bordering the lake, and their inhabitants removed inland. In spite of precautions, however, there is very little doubt that the islanders continued secretly to visit the mainland until they also were removed in 1909, and all the island villages destroyed. Since September, 1909, therefore, there have been no natives in the fly area except the recalcitrant few, who, at the risk of imprisonment, may from time to time return to their old haunts

* Sebwe River is in Toro Province, Uganda Protectorate, in the neighbourhood of Lake George.

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on the mainland or the islands. Instances of this have indeed occasionally occurred up to the time of writing. Apart from the captures made by the Government patrol, I have on several occasions had to report signs of recent native occupation within the prohibited area seen in the course of excursions from Mpumu.

In considering the question of the infectivity of the lake-shore flies, the possibility of infected natives being available as a food supply must still be considered, although this factor is apparently of small importance. These natives would most likely only venture forth at night time, and would be unlikely to approach the fly ground, which is visited almost daily by the boys from Kibanga. There remain the canoemen and fly-boys employed by the Laboratory, and in this connection it can only be said that, although frequently examined, they have never showed any sign of Sleeping Sickness either clinically or upon blood examination.

As all the mainland flies in the following experiments were caught upon a short stretch of shore to the west of the mouth of Buka Bay, which is visited several times weekly, it is extremely improbable that stray natives alone could account for the prolonged infection. The suspicion that either the antelope or reptiles which frequent the lake shore are acting as a reservoir for *T. gambiense* becomes increasingly probable the longer the wild flies remain infective. As to whether reptiles can serve as a reservoir, the difficulty of obtaining live crocodiles or water-lizards has, up to the present, prevented any satisfactory investigations being carried out on this point. That the buck are important in this connection was foreshadowed by results obtained in the laboratory experiments with these animals, some of which have proved capable of infecting laboratory-bred *Glossina palpalis* more than twelve months after their original infection with *T. gambiense*. If an infected buck took up its quarters in the forest behind the fly ground, it might account for the recent outburst of infectivity among the fly after a long period of relative quiescence. From prolonged observation, it would appear that bushbuck, at any rate, among the antelope frequenting the lake shore are very limited in their feeding range.

The objection that these animals would not, by virtue of their nocturnal habits, be exposed to the bites of the fly, may be dismissed at once. On several occasions I have seen bushbuck feeding at the water's edge as late as 8.30 A.M., long before which time the sun is powerful and the fly actively aggressive. Upon one occasion a herd of waterbuck was seen actually in the water at 11 A.M., in the full glare of the sun. Situtunga also, as will be seen later, are by no means so exempt from the attentions of the fly as the general impression of their habits would suggest.

Against the objection that the thick hair of an antelope would prevent a fly from piercing the skin over the greater part of the body, I may say that I have observed *Stomoxys* in numbers vigorously sucking blood on the haunches and sides of the body of a newly-killed bushbuck, regions where the hair is exceedingly thick.

As regards the possibility of the longevity of the fly affording a solution of the problem, I am unaware of any evidence of *G.*

palpalis surviving for more than 227 days—an observation recorded by Dr. Kleine. With a view, then, to completing the chain of evidence incriminating the Lake-shore antelope, the experiments given below were undertaken. Owing to the nature of the country, the expectations of a positive result from the mainland were extremely poor, as the places where buck can be shot are not necessarily places where fly abound. In the immediate vicinity of the fly ground the thick forest absolutely precludes shooting. The results obtained on Damba by Hamerton and Bateman in 1910, and again by Carpenter in May, 1911, showing that the flies on this uninhabited island are apparently maintaining their infectivity undiminished, suggested the scene for the experiments. The only species of antelope found upon the islands of Lake Victoria Nyanza is the Situtunga (*Tragelaphus spekei*). Since the removal of the natives from Damba, these animals have increased greatly in numbers, and at present their tracks may be seen at almost any suitable point on the shore of the island. As will be seen below, events justified the experiment and revealed the parallel in nature to the infections induced in the laboratory.

These investigations include, in addition to antelope, all other available vertebrates whose habits bring them into contact with the fly. In every case where possible the citrated blood was injected into a monkey and a goat, and smears prepared. On rare occasions fresh preparations were examined. Certain mammalian trypanosomes other than *T. gambiense* were obtained in the experimental goats, and these are also discussed below.

The following is a brief summary of the experiments on the infectivity of the wild flies for *T. gambiense*, carried out during the period April, 1911, to January, 1912, covered by the paper:—

Table I.

Date.	Experiment.	No. of flies used.	Result.	Source of flies.
1911.				
April 3–May 10 ...	4	6,144	+	Kibanga.
May 15–28... ..	56	1,050	+	"
June 7–July 18 ...	113	5,385	+	"
July 31–Aug. 31 ...	234	6,360	—	"
Sept. 10–22... ..	386	3,310	—	Lwagi Island.
" 10–22... ..	387	3,250	—	"
Oct. 11–Dec. 5 ...	459	6,150	—	Kibanga.
Dec. 11–27... ..	563	3,190	+	"

Remarks.—Four and a half years after the removal of the natives from the Chagwe coast line, and two years after the final clearing of the neighbouring islands, there is a percentage of 0.014 infective flies in a total of 28,279 caught at Buka Point, assuming that there is only one infective fly to each positive experiment. On Lwagi Island, where there are no antelope, it was impossible to obtain a sufficient number of fly to constitute evidence on the subject of *T. gambiense*. It will, however, be

seen below that there are some grounds for believing that the negative results obtained with Lwagi flies are due to the absence of antelope from this island.

The actual injection experiments will now be considered. Each species of antelope is dealt with in a separate table, the remaining animals being grouped together. A blank space occurring in any column of the tables indicates that there is nothing to record under that heading.

Table II.—Waterbuck.

Date.	Experiment No.	Locality.	Microscopical examination.		Blood injection.				
			Result.	No. of films.	Time after death of animal.	Monkey.		Goat.	
						Quantity.	Result.	Quantity.	Result.
21.4.11...	15	Enfufu			hrs. 2	c.c. 3	—	c.c. 5	—
21.4.11...	15	"			2	3	—	5	—
28.4.11...	26	Kyetume			3	3	—	—	—
24.8.11...	324	Kibanga	—	4	3	3	—	8	—
2.12.11...	544	"			1½	3	—	6	—

Remarks.—With the exception of Experiment 26, all these antelope were shot within the prohibited area and may be considered as more or less frequent visitors to the lake shore.

Table III.—Bushbuck.

Date.	Experiment No.	Locality	Microscopical examination.		Blood injection.				
			Result.	No. of films.	Time of injection after death of animal.	Monkey.		Goat.	
						Quantity.	Result.	Quantity.	Result.
30.4.11...	28	Bugala ...	—	3	hrs. 4½	c.c. 3	—	c.c. 5	—
30.6.11...	183	Kibanga ...	—	6					
30.6.11...	185	"	<i>T. uniforme</i> + + +						
6.8.11 ...	249	Buwera ...	—	3	1½	4	—	10	—
13.8.11...	293	"	—	3	2	4	—	10	—
20.8.11...	312	Ebyoba ...	—		1	3½	—	10	—
2.9.11 ...	353	Kibanga ...	—	2	1	3	—	8	—
27.9.11...	396	Kivuvu ...	—	8		3	—		
19.9.11...	405	Ebyoba ...	—	3					
5.10.11...	435	Namusenyu	—	3	1½	3	—	10	—
20.9.11...	466	Ebyoba ...	—	2	1	3			
19.10.11	472	Namusenyu	—	2	1½	3	—	5	—
21.10.11	479	Ebyoba ...	—	2	1			6	—
22.10.11	481	"	—	2	1½			5	—
22.10.11	482	"	—	2	1			3	—

Remarks.—With the exception of Experiment 396, all these buck were shot within a few hundred yards of the water's edge,

and some actually on the lake shore. In only one case were trypanosomes discovered, *i.e.* Experiment 185, in whose blood an organism answering morphologically to *T. uniforme* was present in large numbers.

Table IV.—Hippo, &c.

Animal.	Date.	Experiment No.	Locality.	Microscopical examination.		Blood injection.				
				Result.	No. of films.	Time after death of animal.	Monkey.		Goat.	
							Quantity.	Result.	Quantity.	Result.
						hrs.	c.c.		c.c.	
Hippo ...	23.4.11	17	Damba ...	—	2	2	3	—	5	—
" ...	4.6.11	106	Namusenyu ...	—	2	3	2½	—	5	—
" ...	11.6.11	132	Kibanga ...	—	2	2	2½	—	5	—
" ...	10.9.11	469	Senkua Island	—	2	3	3	—	10	—
" ...	11.9.11	470	Lwagi Island	—	2	1			10	—
" ...	19.9.11	404	Ebyoba ...	—	2	3½			6	—
" ...	19.9.11	471	" ...	—	2	4	4	—		
" ...	20.9.11	408	Senkua Island	—	2	7			6	—
" ...	24.9.11	409	Damba Island	—	2	3½	3	—		
" ...	24.9.11	406	" ...	—	2	3½	3	—		
" ...	25.10.11	498	Kibanga ...	—		1½	4	—		
" ...	4.11.11	547	Damba ...	—	2	3½	3	—		
Buffalo	4.5.11	37	Near Kyetume	—	4	1½	3	—	5	—
"	4.5.11	38	"	—	4	1½	3	—	5	—
"	11.5.11	49	Kibanga ...	—	4	4	3	—	5	—
"	1.6.11	99	Near Kyetume	—	4	1	2½	—	5	—
"	29.6.11	180	"	—	4	1	2½	—	5	—
"	31.10.11	499	Wankobe ...	—	2	1½	3	—	6	—
Wild pig	1.4.11	3	Kibanga ...	—	2	1	3	—	5	—
Otter ...	4.6.11	109	Namusenyu ...	—	4	2	2½	—	3	—

Remarks.—Experiment 499 was shot far inland. Experiments 37, 38, 99, and 180 were shot on the borders of the prohibited area. It is, however, probable that the herds, in the course of their wanderings, reach the lake shore from time to time, so that they may be considered in the present discussion. All the other animals in Table IV. were shot along the lake edge.

Table V.—Situtunga.

Date.	Experi- ment No.	Locality.	Microscopical examination.		Blood injection.		
			Result.	No. of films.	Time of injection after death of animal.	Monkey.	
						Quantity.	Result.
31.7.11	173	Kibanga	<i>T. uniforme</i> + <i>T. ingens</i> +	∞	Immediate	Several experi- ments.	—
30.6.11	551	"	<i>T. ingens</i> +	4			
10.8.11	284	"	—	6			
16.8.11	302	"	—	3	3 hrs.	3 c.c.	—
22.8.11	317	"	—	5	1 hr.	3½ "	—
28.8.11	332	"	—	3	1 hr.	3 "	—
2.9.11	356	"	<i>T. uniforme</i> +	∞	Immediate	4 "	—
25.9.11	402	Damba Isle	—	3	2 hrs.	3 "	} <i>T. gamb.</i> +
25.9.11	403	"	—	3	1 hr.	4 "	
5.11.11	509	"	—	4	1 hr.	4 "	
6.11.11	510	"	<i>T. uniforme</i> + fresh and stained.		1 hr.	3 "	
						Several experi- ments.	<i>T. vivax</i> + <i>T. uniforme</i> + <i>T. gamb.</i> +

Remarks.—Situtunga Experiments 173 and 356 are still alive at the laboratory.

On numerous occasions trypanosomes have been studied in their blood which agree with *T. uniforme* as regards morphology and movement. Repeated attempts to subinoculate from both these antelope into sheep and goats have, however, up to the present failed. This failure to infect goats with the blood of game known to contain trypanosomes morphologically identical with *T. uniforme* has been already reported by Fraser and myself.

Experiments on these two antelope conducted with laboratory-bred *G. palpalis* show that only the proboscis is infected, the hypopharynx invariably containing a small number of free trypanosomes. This is another point of agreement with *T. uniforme*, which would thus appear the commonest antelope trypanosome of the Mpumu neighbourhood.

The organism referred to above as *T. ingens* in Experiment 173 has only been observed in fresh preparations. It is a large trypanosome with a constant, slow, rippling motion and a very wide undulating membrane. The *T. ingens* of Experiment 551 was seen in stained preparations and answered to the description given by the 1908-10 Commission for this trypanosome.

The reasons for the diagnosis of *T. gambiense* in the above table will be given below. It may be mentioned that Situtunga 173 has developed *T. gambiense* in its blood after being fed upon for five days by flies infected with this trypanosome.

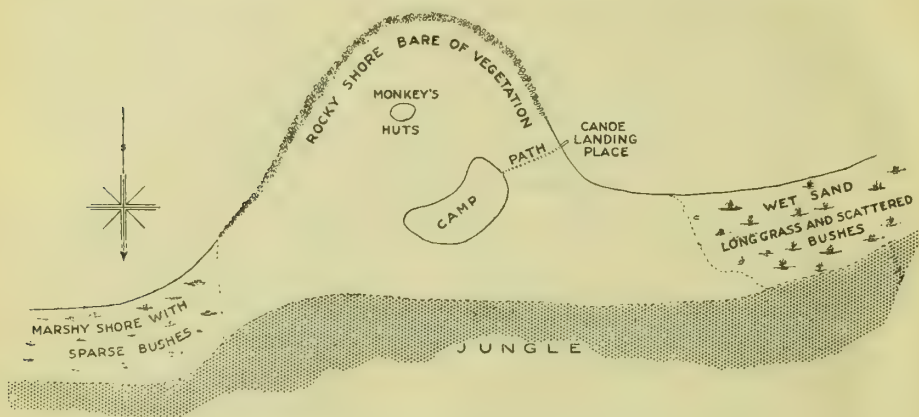
The obtaining of *T. gambiense* from Experiments 402-3 and 509-10 are, of course, by far the most important result so far obtained during the examination of the lake-shore game. It is, therefore, necessary to consider fully all possible sources of fallacy in these experiments and to exclude them as far as possible. Upon this question Dr. Carpenter, at whose camp on Damba Island I made my headquarters, has kindly expressed his views as follows:—

The "Situtunga" Experiment.

"On considering this important result a possible source of fallacy at once suggests itself, namely, that as the monkey was infected whilst on Damba Island, the trypanosomes may have come from a glossina, for I have shown in a 'Progress Report' that the Glossinæ on Damba Island are still capable of infecting a monkey.

"This objection, formidable as it is on paper, is not so when the local conditions are thoroughly appreciated. In the first place, that part of the coast line where is the little promontory on which my camp has been since March is not sufficiently shaded for Glossinæ, which are only present in very small quantity. Moreover, the whole locality round the camp has been very completely cleared, and the edge of the jungle at the base of the promontory,

in which there are few flies, is some 50 yards away from the nearest hut, and 100 yards at least from the monkey's quarters.



"Also, if an occasional fly should accompany the canoe back from that part of the shore where flies are very numerous (nearly two miles away to the south-west) it would not be carried on to the monkey's quarters, but would be intercepted by the boys' huts. As a matter of fact it is an exceptional occurrence to see any Glossinae in the cleared area, and those that are occasionally seen are probably the bred flies which have escaped during manipulations, and these very quickly leave the locality.

"It might be suggested that one of these may have bitten an infected canoeman or fly-boy, and, subsequently, the experimental monkey; but I think it must be an exceptional occurrence for a monkey to be bitten by a glossina. A tame monkey which has been with me on the island since April, and *has never become infected*, is immediately aroused to alertness, even when half asleep, by the buzz of a glossina, and when one of my bred flies accidentally escapes the monkey is all eagerness to catch it, whereas an ordinary fly attracts little attention.

"Moreover, I have no reason to suppose that any of the natives with me on the island are potential sources of *T. gambiense*, and blood from all the fly-boys (who are most exposed to infection) has been injected into a monkey with negative result. •

"(Signed) G. D. H. CARPENTER.

"Damba Island, November, 1911."

It may in addition be pointed out in the case of Experiments 509-510 that both monkey and goat developed *T. gambiense*—confirmed in the latter by sub-inoculation into a monkey as well as in stained preparations; moreover, the goat was tethered in the camp some distance from the monkeys' boxes.

Since May, 1911, Dr. Carpenter has always had two monkeys in residence in the boxes left by Hammerton and Bateman, and

though examined from time to time he has never had a case of spontaneous infection by stray flies. The following is a brief history of the animals which serve as a control to my experiments on Damba Island:—

Monkey A.—Arrived Damba, May 1, 1911, and was immediately used to test infectivity of wild Damba flies. Became infected on May 15, and was immediately shot.

Monkey B (into which the blood of Situtunga 402-3 was injected).—Arrived Damba, May 25, 1911, and between then and September 25, when the injections were made, a period of four months, was examined on 19 occasions, with negative results. On October 2 was negative, and on October 3 Dr. Carpenter first discovered trypanosomes in its blood.

Monkey C.—Arrived Damba, May 29, 1911, and from that date was used by Dr. Carpenter to test the infectivity of wild *G. palpalis* from the neighbourhood of Damba. It was examined on 36 occasions with no result during the ensuing five months. On November 6 it became infected, having by this time been fed upon by several thousands of wild fly.

Monkey D.—Tame monkey kept by Dr. Carpenter, and exposed to all the risks run by the experimental monkeys. Arrived Damba, March, 1911, in perfect health up till October 9, 1911, when, at my request, it was first examined. Since that date has been examined weekly, and has never shown trypanosomes, and is in perfect health.

Monkey E (into which blood of Situtunga 509-10 was injected).—Arrived Damba, October 8, 1911, having previously been examined regularly at Mpumu with negative results. Examined daily from November 3 to November 17 inclusive, with negative results. On November 5 and 6 received blood of Situtunga 509-10, respectively. On November 18 trypanosomes first appeared in its blood.

Monkey F.—Arrived on Damba, November 3, 1911, in perfect health. On November 4 injected with the blood of hippo, Experiment 547 (*cp.* Table IV). Between November 3 and December 12 examined on 21 occasions, with negative results. At present in excellent health.

Finally, it may be pointed out that the incubation period in the case of all three positive experiments was in keeping with previous experience. Thus, Monkey 401 first showed trypanosomes on the eighth day after inoculation. Monkey 511 showed first 13 days after the first injection, and Goat 512 developed *T. uniforme* 10 days, and *T. gambiense* and *T. vivax* 15 days after inoculation. With reference to the prolonged incubation period in the case of Monkey 511, it may be noted that, in the only case where an inoculation from the laboratory antelope has recently proved positive, the incubation period in the monkey before trypanosomes appeared was 18 days.

A consideration of the conditions on Damba Island affords a vivid picture—a vicious circle in constant operation, only awaiting the reappearance of the natives to reproduce the recent epidemic.

With the exception of papyrus areas, practically the whole shore line of the island is to a greater or less extent fly-ridden, one portion being occupied by the famous fly-beach, from which for several years some thousands of pupæ have been brought monthly to Mpumu. In his recent report to the Royal Society, Carpenter describes an infection of a monkey with 885 flies caught in May, 1911, on this pupa ground. The situtunga are multiplying rapidly in the absence of the natives, and are far less restricted in their range than formerly. The common conception regarding the habits of these antelope, as a creature spending its days in the papyrus swamps where no fly are found, and only visiting dry land at night to feed, does not apply to Damba. During the short time I spent on the island, I frequently saw situtunga from the canoe at the water's edge both morning and evening. As late as 9 A.M. I have seen them feeding among the ambatch stems in places where fly were numerous, and where it required constant vigilance to avoid being bitten. On one occasion I observed a female on the actual pupa-beach at 8.30 A.M. in the full glare of the sun. There is also a long extent of shore, where, owing to comparatively recent sinking of the lake level, there is now a strip of grass and bushes some 40-50 yards wide, dotted here and there with ambatch trees, between the forest and the water's edge. This is a favourite haunt of the situtunga, and tsetse are present throughout its extent. There is therefore every natural facility for that frequent interchange of infection between fly and buck which constitutes a true reservoir.

On the neighbouring coast of the mainland, the fly are probably brought into far less frequent contact with antelope. The stretches of shore where fly are numerous are few and far between, and the number of antelope which actually visit any given fly area would be small. On the mainland also, the situtunga would probably only be available to the fly residing in the immediate neighbourhood of the papyrus, and thus the most typically Lake-shore antelope would be denied to the fly where the latter are most numerous.

Finally, the following table may be considered as supplying additional evidence on the part played by antelope as a trypanosome reservoir:—

Table VI.

Date.	Experiment No.	No. of flies used.	Result.	Source of fly.	Experimental animal.
1911.					
May 16-24 ...	58	1,100	<i>T. gambiense</i> +	} Kibanga ...	Goat.
July 24-Aug. 2	223	1,220	<i>T. uniforme</i> +		
Sept. 27-Oct. 4	400	1,560	<i>T. uniforme</i> +		
„ 9-22 ..	384	4,258	<i>T. vivax</i> +	} „ ...	„
			—		
				Lwagi Island	„

Remarks.—It will be seen from this table that in the first three experiments, comprising in all 3,880 mainland flies caught at

Buka Point, *T. uniforme*, with or without *T. vivax*, appeared on all three occasions. A total of 4,258 flies caught on Lwagi Island, where there are no antelope, but only hippo, birds, and reptiles, failed to produce either of these trypanosomes.

Diagnosis of the Trypanosome Obtained in Monkeys 401 and 511.

There seems no reason to doubt that the trypanosome obtained in monkeys from the blood of Situtunga 402-3 and 509-10 is identical with the species obtained by Hammerton and Bateman from wild Damba flies in May-June, 1910, and again in May, 1911, by Dr. Carpenter. The Damba natives suffered severely during the epidemic, as the conditions on the island ensured constant exposure to the bites of the fly. These fly are still infective to monkeys, and the trypanosome carried by them answers to *T. gambiense* morphologically, and as regards the disease in monkeys.

In the face of such facts a more comprehensive examination seemed hardly necessary. In the present instance, however, the importance of the issue at stake makes a careful investigation imperative.

Of the trypanosomes previously described from Uganda *T. brucei* is the only one which claims attention. Other species as *T. pecaui* and *T. "dimorphon"* are also suggested by the morphology. The fact that *T. brucei* is supposed to be non-pathogenic to man emphasises the importance of excluding this species in diagnosing a trypanosome derived from wild antelope.

Behaviour of the Damba Trypanosome in Glossina palpalis.

In an experiment comprising 101 clean laboratory-bred *G. palpalis* fed upon Monkey 401, whose blood contained the Damba trypanosome, two infected flies were obtained on the 17th and 57th days respectively.

The clean monkey upon which they were fed developed trypanosomes answering to *T. gambiense*. The 57th day fly showed a typical *T. gambiense* infection of gut, proventriculus and salivary glands, the proboscis being negative. This latter fact would point to the exclusion of *T. pecaui* and *T. dimorphon* from the differential diagnosis.

A second experiment, in which 51 laboratory-bred flies were used, proved negative.

An experiment comprising 79 laboratory-bred *G. palpalis* fed upon Monkey 511 gave one infected fly on the 42nd day. The gut only contained trypanosomes. The clean monkey upon which these flies were fed ran away without having ever shown trypanosomes.

Morphology.—Dimorphism marked, the extremes 35μ and 17μ . The long or short type may predominate from day to day, but intermediate forms are always present. Forms over 31μ in length are very uncommon; the longest examples were met with in Experiment 504, a monkey infected from Experiment 401, through laboratory-bred flies.

The following table shows the distribution of length in 10 experimental animals:—

Table VII.

Origin of trypanosome.	Experiment No.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	Average.
Situtunga 402-3	...		2	2	1	4	1	2	1			3	1	1		2	1				24.4
" "	...		2	2	2	1	1	1	1	2	3	2	1	1		1		1	3	2	24.3
" "	...	2	1	1	1	2	1	2	1	1	2	2	1	2							27.4
" "	...		1	1	3	5	2	2	1	1	1	2	2	4							23.7
" "	...		1	8	1	1	2	2	1	2	1	3			1	1	1	1			20.1
" "	...			1	3	3	5	3	1	1	1	2									25.6
" (1) 511, monkey	...		1	1	3	5	6	2	2	1	1	2	1		1						22.35
" (2) 511, "	...		1	2	1	2	1	4	1	4	2	2	1								22.1
" 525, "	...			2	1	2	1	1	1	3	1	1	3								23.5
" 574, dog	...			1	1	2	1	3	4	3	1	1									23.9
Totals ...	...	2	7	19	13	24	19	26	12	14	12	18	9	9	3	4	2	2	3	2	
Percentages ...	...	1	3.5	9.5	6.5	12	9.5	13	6	7	6	9	4.5	4.5	1.5	2	1	1	1.5	1	

The *Protoplasm* is devoid of granules in all preparations examined from goats and monkeys. In both the rats examined

granules were present in numbers both behind and in front of the nucleus.

A free portion is always discernable in the flagellum, though, in the case of the stumpy forms, it is often extremely short.

Movement.—No marked translation; remains actively wriggling in one spot, making occasional short gliding excursions about the field. Often the side-to-side movements are of a jerky character.

Table VIII.—Animal Reactions.

Animal.	Expt. No.	Source of virus.	Incubation.	Duration.	Remarks.
Monkey...	401	Situtunga 402-3	days. 8	days.	Alive after 124 days; shows considerable emaciation; no lethargy.
" ...	511	" 509-10	12-13		Alive after 82 days; no obvious symptoms.
" ...	504	Flies from 401	?		Alive after 75 days; in good health.
" ...	525	Goat 512	10		Alive after 70 days; in good health.
" ...	543	Calf 478	10		Alive after 54 days; in good health.
" ...	575	Goat 579	6		Alive after 42 days; in good health.
Rat, white	477	Monkey 401	5	83	Alive after 42 days; slight emaciation; lively.
"	571	"	5		
Guinea-pig	458	"	10	61	Alive after 42 days; apparently in excellent health.
"	572	Rat 477	20		
Goat ...	512	Situtunga 509-10	15(?)		Alive, and apparently in good health, after 82 days.
" ...	579	Monkey 401	?		Never showed trypanosomes, proved by sub-inoculation into monkey.
Dog ...	573	"	12		Alive after 90 days; well.
" ...	574	Goat 512	14		Alive after 42 days; in good condition.
Calf ...	478	Monkey 401	?		In very poor condition at time of inoculation and during the experiment.
					Proved by sub-inoculation into monkey. Trypanosomes seen on one occasion 51 days after inoculation. No obvious symptoms after 99 days.

These experiments though few in number afford valuable evidence in favour of *T. gambiense* to the exclusion of *T. brucei*. The sub-chronic type of the disease in rats is perhaps the most striking point in the above table.

Action of Human Serum.—The assumption that *T. brucei* is non-pathogenic to man rests chiefly on the susceptibility of this trypanosome to human serum. Laveran and Mesnil,* in describing the action of this serum on animals infected with *T. brucei*, confine their attention chiefly to rats. The following experiments

* Laveran and Mesnil 'Trypanosomes et Trypanosomiasés,' 1904.

Remarks.—It will be seen that in every case where the human serum was injected into a monkey the trypanosomes disappeared from the circulation for a longer or shorter period. In the case of the rats this did not occur. It would thus appear that there is some condition present in monkeys which causes them to react to human serum, this condition not being present in rats. Experiments conducted *in vitro* on the same lines appear to confirm this conclusion. The negative results obtained with Rats 477 and 576, together with the positive result obtained with the human strain in Monkey 391, neutralises what at first appeared evidence in favour of *T. brucei*.

As regards the diagnosis of the three species of trypanosomes described in Goat 512 (Table V), the following methods were employed:—

(1.) Examination of fresh blood preparations.

By this means of *T. uniforme* was identified in the blood of Situtunga 510, and in the case of Goat 512 all three species were recognisable.

(2.) Examination of stained films.

T. uniforme identified in blood of Situtunga 510.

maxim., 19.00 μ ; minim., 14.5 μ ; average, 15.9 μ (dry fixation).

T. vivax in Goat 512.

maxim., 27.0 μ ; minim., 20 μ ; average, 23.9 μ (osmic acid fixation).

T. gambiense in Goat 512.

T. vivax and *T. gambiense* in centrifuged blood of Goat 512.

(3) Sub-inoculation.

The blood of Goat 512 inoculated into Monkey 525 produced *T. gambiense* in the blood of the Monkey.

Conclusions.

(1) That the continued infectivity of the wild *G. palpalis* on Damba Island to monkeys may be explained by the fact that the situtunga on that island are acting as a reservoir to *T. gambiense*.

(2) That the continued infectivity of the mainland flies to *T. gambiense* may probably be explained on a similar hypothesis, including possibly the other species of antelope frequenting the Lake shore, *i.e.*, waterbuck, bushbuck, duiker and reedbuck.

(3) That no positive evidence can be adduced from the above experiments to show that hippo can serve as a reservoir.

(4) That the continued infectivity of the Lake-shore flies to *T. vivax* and *T. uniforme* is also due to the antelope, which serve as a reservoir for these trypanosomes.

DESCRIPTION OF PLATE 30.

- a. *T. uniforme* from blood of Situtunga 511. Fixed by drying only followed by absolute alcohol. ($\times 2,000$, and so for other forms.)
- b. *T. vivax* from blood of Goat 512. Fixed with osmic acid followed by absolute alcohol.
- d. *T. gambiense* from blood of Monkey 401, which was infected from Situtunga 402-403. Fixed as last.
- e. *T. gambiense* from blood of Monkey 504, infected by laboratory-bred glossinæ from Monkey 401. Fixed as last.
- f. *T. gambiense* from blood of Rat 477, infected from Monkey 401. Fixed as last.
- g. *T. gambiense* from blood of Monkey 511, infected from Situtunga 509-510. Fixed as last.

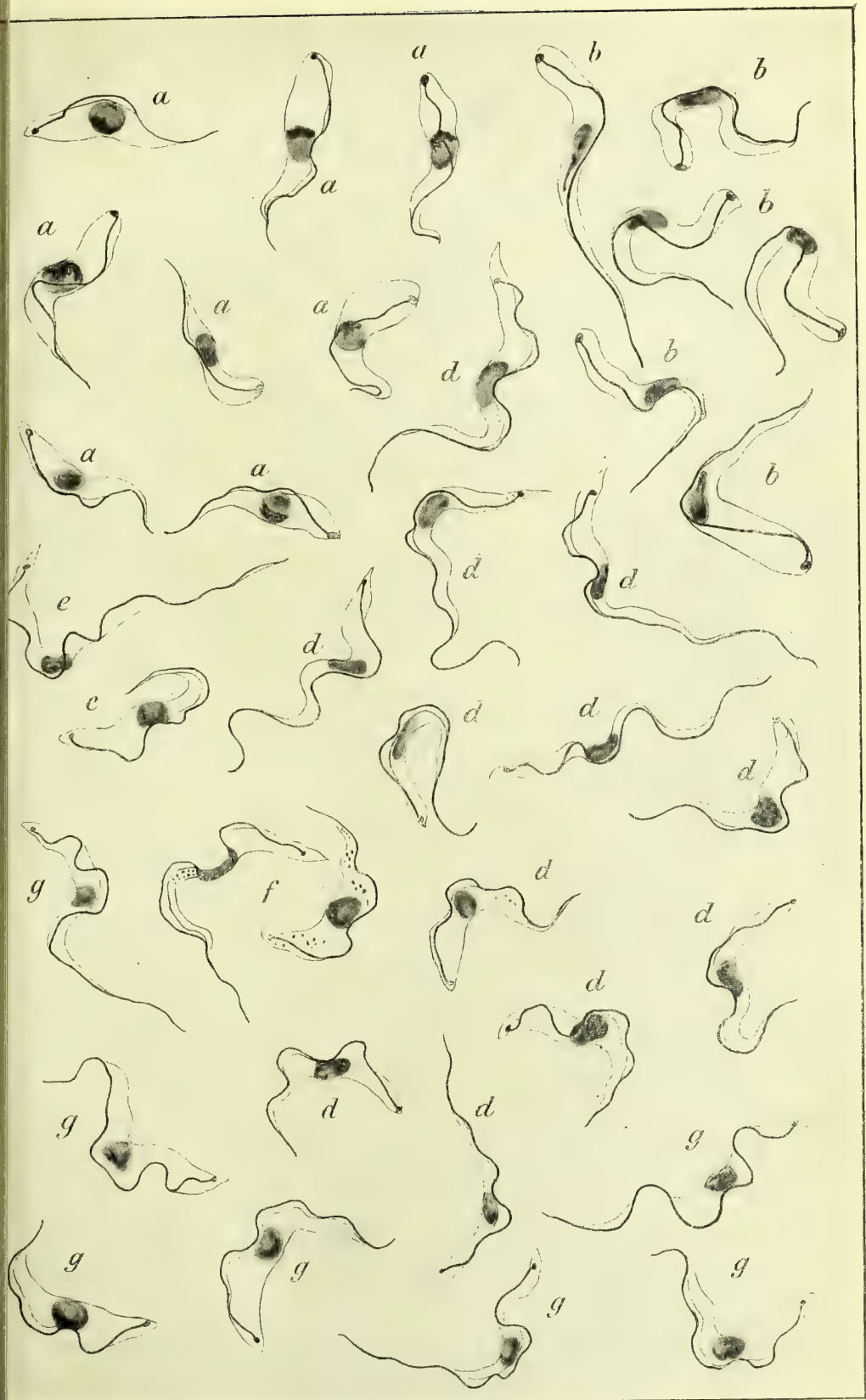
15.—NOTES ON SOME FLAGELLATE INFECTIONS FOUND IN CERTAIN HEMIPTERA IN UGANDA.*

By MURIEL ROBERTSON.

While carrying out other work on Mpumu, the opportunity has presented itself of examining the Hemiptera from the surrounding country, and some interesting protozoan infections have been found. The group has a certain importance in this connection in that it includes the two parasitic genera, *Cimex* and *Conorrhynus*. *Cimex* has fallen under suspicion in regard to kala azar, and a species of *Conorrhynus* is definitely incriminated as the transmitting agent of the South American trypanosomiasis. Certain non-parasitic species, generally belonging to the group of the Reduviidæ, occasionally attack man. An instance of this has been reported by Dr. H. L. Duke from the neighbourhood of Mpumu. He has on several occasions been bitten by a hitherto unrecorded species of *Henicocephalus*. Cases of this kind are also known from other parts of Africa, and from India. It is, therefore, not without interest to obtain some knowledge of the Protozoa infesting Hemiptera generally, and more especially of the flagellates. So far as I am aware none of the species dealt with here are known to attack man or other vertebrates.

The parasites considered belong almost exclusively to the herpetomonad or crithidial type. I do not propose to attach species names, as the cycles have not been worked out, and the nomenclature is already in a very confused state. There is at present some considerable difference of opinion as to what is the correct name of the genus, and as none of the literature is accessible this question is not touched upon at all. In every case the specimens I have examined show a single broad flagellum, and I have used the term herpetomonas to describe all forms without an undulating membrane, crithidia for forms with a membrane, and with the kinetonucleus anterior to the trophonucleus. The word crithidia is not used in any generic sense, but simply as a convenient term to denote flagellates showing the above-mentioned relation of membrane, kinetonucleus and trophonucleus.

* Reprinted from Proceedings of the Royal Society, Series B, vol. 85, 1912.



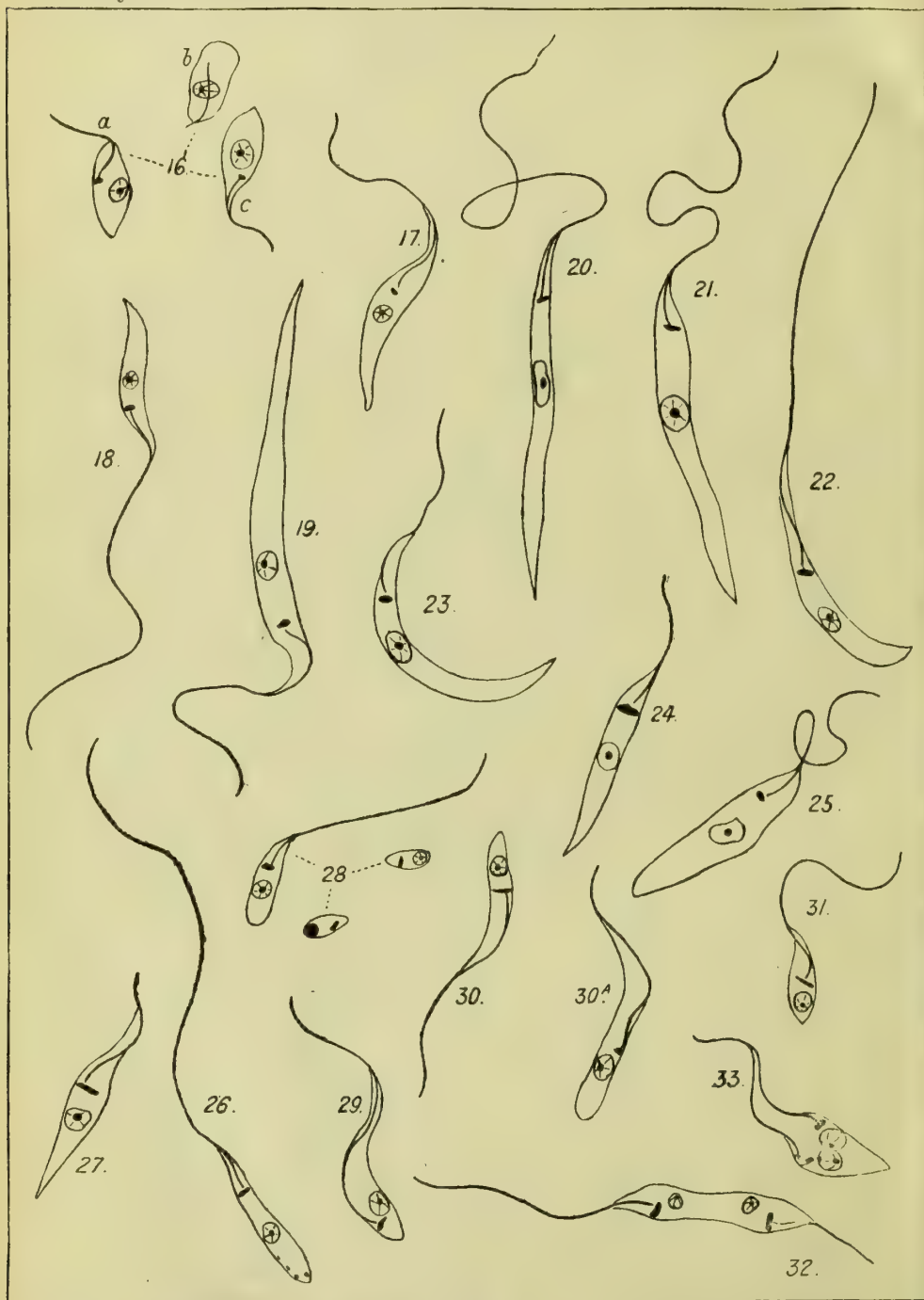


Several of the insects examined have, as will be seen later, shown a confusing range of forms, whose real connection cannot be ascertained with any satisfactory degree of certainty by the



mere examination of a relatively small number of natural infections. There is, therefore, every reason why species names should be withheld until these points are settled beyond doubt.

All the material was fixed by the wet method, using Schaudinn's fluid, and stained either by Heidenhain's hæmatoxylin or by Mayer's hæmalum.



Leptoglossus membranaceus Fab. (I) is a common Uganda species; it feeds on a certain gourd-like creeper, showing a marked preference for this particular plant, and is hardly ever found

except in its vicinity. The specimens are frequently infected in the gut with a long slender herpetomonas, figs. 1-7 give an idea of its general characters and appearance. The gut forms range from broad sac-like creatures with a short flagellum (fig. 7) to long slender forms with a flagellum of great length (fig. 1). The nucleus in the broad forms is of the type so common in trypanosomes and many other flagellates, and is made up of a large karyosome surrounded by a clear space, the outer limit of which is marked off by a delicate membrane; between the karyosome and the membrane there pass fine radiating strands. In the more slender forms there is to be noticed a lengthening out of the nucleus and a tendency for the karyosome to fragment (figs. 2 and 3). Finally, in the very long types the nucleus is drawn out into a very narrow and much elongated oval, the karyosome being represented by a row of chromatin blocks of slightly irregular shapes (fig. 1). I have not been able to detect any sign of the fine strands between the karyosome and the membrane in this phase of the nucleus. The body of the long herpetomonads is markedly flexible, which is a somewhat unusual feature in parasites of this type.

Although there is no direct evidence as regards the sequence of forms in the material handled, there is little doubt from all the work done on this type of protozoon, that the long slender creatures may be regarded as the adult individuals, and that they have been produced from the smaller broad forms. In all the infected specimens of *Leptoglossus* so far examined, I have not come across any of the curious crithridial forms which are so characteristic a feature of two types of infection to be discussed later.

Quite the most important point in the infection of *Leptoglossus membranaceus* is the fact that, in adult specimens, the salivary glands are very frequently strongly infected with the flagellates. Young specimens show the parasites only in the gut, and, as in the histories of most parasitic flagellates, the infection seems to work forwards, from the hinder part of the gut near the Malpighian tubules. The flagellates are not present in large numbers in the anterior part of the intestine in any of the specimens so far examined. The individuals seen in the salivary glands show a considerable range of shape and size, and curious small forms are present in fair numbers. It is not proposed to enter into these relations in detail, as this herpetomonas will, I hope, be the subject of more systematic study later on.

In the infection of the salivary glands of *Leptoglossus*, we have the independent development in a sucking insect of all the factors requisite for the transmission of a flagellate, parasitic in the intestine, by way of the mouth-parts of the insect host. This is a significant step in the history of how the complex interacting circumstances which we now find in operation in such diseases as trypanosomiasis, kala azar, and malaria may have arisen in nature. I have not so far found the parasite in the plant, but this part of the work has not been carried anything like far enough as yet to permit of a statement being made in regard to the point. These conditions in *Leptoglossus* are not at present of the slightest practical importance, but I should like to point out that it has a certain interest to find this state of affairs in Uganda involving a parasite

of the same genus as that which produces the diseases of kala azar and Oriental sore.

Leptoglossus occasionally shows an infection with a coccidian, which I do not propose to describe, but there is one point that bears on the matter under discussion. This coccidian also invades the salivary glands, but probably does it by way of the body cavity. Cysts containing sporozoites were found in the salivary gland and in the gut, as also all the stages of schyzogony or asexual multiple fission. Motile, sickle-shaped individuals were found in the proboscis: morphologically, there is nothing in the live state to distinguish sporozoites and young merozoites, the probability is, however, strongly in favour of their being merozoites. I am inclined to think that the presence of these forms in the proboscis has no significance in the coccidian cycle, but here again the condition is not without its reference to the question of the phylogenetic development of parasitic protozoa. The close relationship between the *Hæmogregarinæ* and the *Coccidia* is now well established, not to mention the general parallelism in all essential features between the cycle of the malarial organism and that of any typical coccidian. Not to lay undue weight on these points, the main feature here, as in the previous instance, is the arrival of an intestinal parasite in the mouth-parts of a sucking insect, which is, however, still a plant sucker.

The concurrence of the flagellate and coccidian parasites in the *Leptoglossus* and the fact that they have reached the salivary gland in both cases is rather striking in view of the frequent association of flagellates and *Hæmogregarines* in the blood stream of vertebrates.

Dysdercus casiatus (XIV), the red cotton-bug, shows an occasional infection with a typical herpetomonas; so far the parasites have only been found in the gut. Only a small number of infected specimens have been examined, figs. 8-10 show the general characters of the parasites; no crithidial forms have been found in these infections.

Carbula jipensis (X).—This small bug shows rare and generally slight infections with a herpetomonas.

Two sets of infections (one in a reduviid XV, the other in a capsid bug V) now come to be considered which differ from those hitherto handled in that crithidial forms are present in large numbers as well as the typical "needle-and-thread" herpetomonads and the broad forms from which they spring. It is almost impossible to come to a really satisfactory decision as to whether there are two different species present or whether all the forms represent different stages of one species. I am inclined to consider, however, that in each of the two cases one is dealing with a single parasite, and that the crithidial forms, though sometimes present in large numbers, are more or less transitory growth forms, and that the slender herpetomonads (11, 20, and 21) are the adult individuals. Nevertheless, the opposite view could be so well defended and has so much in its favour that it must be considered as practically an open question until more evidence is to hand.

The facts that, in my opinion, weigh the scale in favour of one species in which the long herpetomonads are the adults and the

crithidia transitory growth forms are as follows, and apply equally to either of the two sets of infections:—

1. The herpetomonad infections are sometimes found unaccompanied by the crithidial forms, but the converse has never been observed.

2. The crithidial forms are generally present in strong, actively, multiplying infections.

3. The crithidial forms merge into the herpetomonad types.

The appearance of the crithidial forms is probably due to the rapid growth of the flagellum, which is finally very long, causing the displacement of the kintonucleus. The flagellum is in any case attached to the periplast for part of the way, and the development of the membrane in these forms is never more than a very narrow ridge of periplast along which the flagellum runs. It looks as though when the body is lengthened out this uneven growth were compensated and the herpetomonad type was reached, partly by the drawing out of the posterior end of the body, and partly by the moving forward again of the kintonucleus.

Many herpetomonad infections show this confusion of types, for instance that of the house-fly in Europe and also in India. No work has been done on these forms which has clearly shown the connection of these various forms one with another. In the house-fly the difficulties in the way are very great, and the life-histories of herpetomonads seem to be in need of further study. It is hoped that the form in *Leptoglossus* may be worked at in greater detail, as this bug breeds readily in captivity and is easily fed.

The first of the infections just discussed occurs in a bug belonging to the family of the Capsidæ (V); it is found in or near forest and is of the same light brown colour as the fallen leaves on which it runs. Its food is not known and it is apparently not a very common bug. Figs. 11-19 illustrate the main types found in this infection.

The second of these infections was found in a red and black reduviid (XV) which has not as yet been identified. It is a common species and feeds on small bugs and on other insects; the specimens are frequently infected, but I have not so far found any showing the flagellates in the salivary glands. The herpetomonas is small in size, and some of the infections, notably those in which the crithidial forms are numerous, show large numbers of small rounded forms without a flagellum. Figs. 20-33 show the features of the parasite more clearly than any mere verbal description.

I am indebted to Mr. Gowdey, the Government entomologist, for kindly identifying the species named.

16. NOTES ON CERTAIN ASPECTS OF THE DEVELOPMENT OF *TRYPANOSOMA GAMBIENSE* IN *GLOSSINA PALPALIS*.*

By MURIEL ROBERTSON.

In the course of an attempt to obtain an insight into the details of the life-cycle of *Trypanosoma gambiense* in *Glossina palpalis*, certain experiments were undertaken involving the feeding of a relatively large number of flies under closely observed conditions. Although primarily undertaken with a view to the morphology and development of the parasite, they have a bearing on the general relation between the trypanosomes and the *Glossina* that is of some interest.

The present account deals with the infections produced in the flies as a whole; the morphological results will be considered in detail elsewhere. I must point out that the experiments in question are not concerned with actual transmissions of *T. gambiense* from an infected to a clean host, but with the number of flies in which the trypanosomes will develop. That flies harbouring trypanosomes are infective from about the 26th day onwards has been shown over and over again; it was therefore considered to be a wanton waste of life to allow every cage kept beyond the 30th day to infect a clean animal. Late cages were usually fed on cock's blood after the 24th day. A small proportion were actually tested and an infection was invariably produced if the box contained flies showing trypanosomes.

There is no evidence to show that a trypanosome-infection once established in the fly is ever got rid of subsequently. *T. gambiense* may be held to be established if the gut shows trypanosomes after the 5th day in flies which have had *at least one feed of clean blood* subsequent to the infecting feed.

This last statement bears on a point of some importance; it has been found during the course of these experiments that flies allowed to have one infected feed and then starved absolutely, when dissected between the 6th and the 12th day, show an extraordinary number of individuals in which trypanosomes are to be found. Flies starved in this way rarely live beyond the 12th or 13th day. These experiments will be referred to as starvation experiments.

Of 103 flies so treated and fed (for one infecting feed only) in groups on different monkeys infected with *T. gambiense*, 22 showed trypanosomes between the 6th and 12th day, that is to say 21·3 per cent. of the flies harboured trypanosomes. Six flies of the total 22 showed trypanosomes only in the sucking stomach, or crop, as this organ should perhaps be more appropriately called, 13 showed a well-established infection in the gut and three showed trypanosomes in both situations. It is perhaps advisable to neglect the six flies in which the parasite was only present in the crop, although a certain amount of development may go on in this organ; the percentage thus obtained is still very high, namely, 15·5.

* Reprinted from Proceedings of the Royal Society, Series B, vol. 85, 1912.

Monkeys infected with *T. gambiense*, and probably most other animals with trypanosomes in their blood, have negative periods, that is to say periods during which they do not infect flies. A number of the experiments have shown that trypanosomes may be found by microscopic examination, although the blood is not infective to flies. It is interesting to note that such negative periods appear to be negative in starved as well as in fed flies. This relation is, however, not yet sufficiently worked out. In the starvation experiments the microscopic appearances do not, so far as I have yet seen, show any distinction from those to be observed in established infections in fed flies of the corresponding ages.

The crucial moment in the cycle appears to be the first feed of clean blood subsequent to the infecting feed. It is not evident if this clearing out of the trypanosomes by the clean feed is a purely mechanical action due to the flooding of the gut or is a result of the general change of condition thus brought about.

In any case the number of flies containing trypanosomes obtained in starvation experiments during periods when the vertebrate is in the infective condition would give the maximum register of the potential infectivity of that individual strain to fly. The actual number of flies containing trypanosomes from parallel experiments, which were, however, subsequently fed, would give an indication of the additional inhibiting power of the fly under ordinary conditions whatever the cause to which the inhibition may be due. Experiments of this type were undertaken but gave no result, as the whole series proved negative, the monkey not being in the infective condition.

The total number of flies used in the whole group of experiments under consideration in this paper is 1,411 males and 1,322 females, of which 42 males and 39 females show trypanosomes. Irrespective of sex, the total number is 2,733, of which 81 gave a positive result.

From this total must be deducted the starvation experiments and a small group which are not strictly comparable, owing to the feeding having included toad's blood, and also those flies dissected before the 5th day. Concisely, the figures stand thus:—

Total	2,733 flies; infected flies, 81.
Toad and starvation experiments.	234 flies; infected flies, 17.
Flies dissected between days 1-5..	84 flies; infected flies, 9.

Deducting the last two batches from the total there remain 2,415 flies, of which 55 were infected; that is 2·27 per cent. of the flies harboured trypanosomes.

This percentage, *i.e.*, 2·27, is naturally not the measure of the infectivity to the fly of any strain (or strains) of trypanosome. It is the percentage of infected individuals produced by allowing 2,415 flies to feed at random, in groups, through a period of two and a half months, on a population of nine infected monkeys. Each group receives, of course, only one or two feeds on the infecting monkey, and is then fed on clean animals.

Certain obscuring features, habitually neglected in dealing with trypanosome infections, must be pointed out in figures handled in this way. The nature of the individual strain must be considered,

and the occurrence of negative periods (*i.e.*, periods when the vertebrate is not infective to fly) must be duly taken into account. As they stand, the figures above cited have no real meaning. The number of infected individuals obtained by feeding flies at random upon an infected monkey or other vertebrate is neither an index of the infectivity of the strain nor of the potential danger of such an animal at large in a fly area. The percentage, however, of infected individuals produced among flies fed during periods when the blood is infective, gives the index of the virulence of the strain as regards fly. If on the other hand, batches of, say, 50 or 100 flies were fed on an infected monkey for every day of its life during the course of the disease, the infected glossinæ thus produced would give an index of the infective power of the monkey as a whole.

It is obvious that there are two quite different aspects of the question, and calculations in which they are treated as one must naturally be misleading. In practice it seems usual to neglect this distinction, with the result that there has been a tendency to underestimate the potential transmitting capacity of the fly, and to overrate its individual idiosyncrasy. Given reasonably favourable conditions of temperature and moisture, it is the strain of trypanosomes and not the fly that within a relatively wide range plays the deciding rôle in limiting the number of infected glossina. There is, of course, as has already been mentioned, a serious difficulty in the way of the trypanosome in its attempt to establish itself at all in the glossina, but that must be very nearly constant in all cases.

To consider some of the experiments in greater detail:—Monkey 113, infected by wild flies from the lake-shore, first showed trypanosomes in its blood on July 25, 1911.

On August 23, Monkey 113 showed trypanosomes in its blood; 137 flies were fed in groups, 36 of these were treated as a starvation experiment; the whole series proved negative with the exception of one starved cage, which showed one infected fly on the 12th day.

On August 24, 45 flies were fed on the same monkey, and there resulted five infected flies, that is, a percentage of 11.1 showed trypanosomes.

On August 25, 53 flies were fed, of which two were infected, which is equal to a percentage of 3.7.

On August 26, however, 89 flies were fed in three groups on this same monkey, and produced no infected flies at all. The experiments ran concurrently, and shared the same weather and other external conditions, and were similarly fed. Here, as all through this paper, results of flies dissected before and on the 5th day are excluded. In the experiments just mentioned the flies were dissected at different periods, but all after the 20th day. One is forced to the conclusion that, although the monkey showed trypanosomes on all the four days in question at the time of feeding the flies, the blood was only very slightly infective on the 23rd, probably not at all to fed flies; that on the 24th and 25th it was infective, producing (adding the results for the two days) seven infected individuals out of 98 flies, *i.e.*, 7.1 per cent.; and that it was once more non-effective on the 26th. Under conditions

so similar it is impossible to consider a difference of 7.1 per cent. to be due to the individual variations of the two laboratory-hatched sets of flies. The onus of this discrepancy must obviously be borne by the trypanosomes derived from the monkey.

It is interesting to note in this connection the result of microscopic examination of the live blood of this monkey over these and the adjacent days.

Table I.—Monkey 113.

Date.	Condition of blood examined alive.	+ or — laboratory flies.	Number of flies fed.	Percentage of + flies.	Number of experiment.
Aug. 20	No trypanosomes seen in blood.	No flies fed	—	—	—
" 21	" "	"	—	—	—
" 22	" "	"	—	—	—
" 23	Few " "	—	101	—	38-42
" 24	Numerous trypanosomes seen in blood.	+	45	11.1	45 and 45
" 25	" "	+	53	3.7	46
" 26	Very numerous trypanosomes in blood.	—	89	—	47-49
" 27	Very few trypanosomes seen in blood.	No flies fed	—	—	—
" 28	} Showed no trypanosomes in blood. }	—	25	—	50
" 29		(flies fed both days)	—	—	—
" 30	Shows few trypanosomes	—	34	—	55 and 56
" 31	" "	+	107	1.8	54
		(box fed on 30th and 31st)			
Sept. 1	} Not examined.				
" 2					
" 3					
" 4	Very few trypanosomes ...	—	32	—	58
" 5	" " " ...	No flies fed	—	—	—
" 6	Fair number of trypanosomes; dimorphism of broad and narrow forms very marked.	—	49	—	60
" 7	Fair number of trypanosomes.	No flies fed	—	—	—
" 8	Trypanosomes very numerous indeed.	+	95	2.1	62 and 64
" 9	Fair number of trypanosomes, but much fewer than on 8th.	No flies fed	—	—	—
" 10	Not examined.				
" 11	Few trypanosomes ...	No flies fed	—	—	—
" 12	Trypanosomes very numerous indeed.	+	70	1.4	68
" 13	" "	No flies fed	—	—	—
" 14	" "	—	—	—	—
" 15	Blood swarming with trypanosomes.	+	20	10.0	75

For the sake of completeness I add the percentage of infected flies produced over all the boxes containing infected flies obtained

from Monkey 113. This includes some results from dates earlier than those of the foregoing table. Starvation results and flies dissected before the fifth day are as usual excluded.

			Flies.	Infected.
Experiment	25	...	69	2
"	29	...	60	1
"	32	...	71	1
"	43	...	32	3
"	45	...	13	2
"	46	...	53	2
"	54	...	107	2
"	64	...	104	2
"	68	...	70	1
"	75	...	20	2
Total			599	18

The percentage of infected flies is equal to 3 per cent. I do not add the percentage obtained by including all the boxes in which the result was negative. The number so obtained would be a mere numerical curiosity, as, with our present knowledge, it is purely a matter of chance from the point of view of the experimenter, how often he happens to strike a non-effective period. It is obvious that a quite uncontrollable factor is introduced if the figures are so handled. It becomes clear that although a trypanosome-infection is in a continual state of flux the percentage of plus flies produced over infective periods gives a measure of the virulence of the strain to fly and forms a basis of comparison between different strains.

Another set of experiments bears on this point. Monkey 199 was infected by Dr. Duke by direct injection of blood from a bush-buck which had been infected with *T. gambiense* by laboratory infected flies. The bush-buck had harboured *T. gambiense* for 15 months. This monkey showed infective and non-infective periods in exactly the same way as other infections, but the infective periods gave quite an unusual number of flies harbouring trypanosomes. Thus—

Experiment 71, 13/9/11, 54 flies fed, gave 4 positive, = 7·4 per cent.
 „ 70, 14/9/11, 50 „ „ 7 „ = 14·0 „
 „ 74, 15/9/11, 46 „ „ 5 „ = 10·8 „

Considering all the figures together, out of 150 flies, 16 showed trypanosomes, that is a percentage of 10·6 per cent.

This relatively very high percentage was also borne out in experiments of Dr. Duke's in which he made use of this monkey and which he kindly permits me to quote. Thus of 188 flies from two experiments the conditions of which admit of comparison with those of Experiments 71, 70, and 74 just cited, 11 were infected, that is 5·8 per cent.

Taking this set of figures with those quoted above, of 338 flies, 27 were infected, which is equal to a percentage of 8 per cent. This is more than double the normal percentage of infected flies

produced by the Uganda strain of *T. gambiense* in monkeys. All the conditions being considered, it is impossible to attribute this difference to anything but the strain of trypanosomes.

Besides having a virulent character as regards the production of infected flies as a whole, an individual strain has often a recognisable type or method of development in the glossina. For instance, all the flies fed on Monkey 199 gave very numerous and rapidly developing infections; the trypanosomes reached the proventriculus earlier in the cycle than is usual, and were established in the salivary glands much more promptly than in the case of ordinary cycles. One cage was infective on the 24th day. This difference of character appeared in the flies from Dr. Duke's experiments, as well as in those cited above, and I am indebted to him for the opportunity of examining them. The monkeys infected by fly fed on Monkey 199 showed good infections in the blood (Monkeys 330, 390, and 391), but flies fed on these monkeys gave only an average number of infected flies, *i.e.*, 3 per cent., which, however, showed rather sluggish and very slowly developing infections. Thus, one of the cages fed on one of these monkeys showed an infected fly, in which the infection had not yet reached forward beyond the mid-gut on the 22nd day, and another on the 56th day, in which the salivary glands were not yet infected. There was no possible chance of a "pick up" infection in either case. A stray fly, showing a very backward infection, is generally due to having allowed a cage to stay too long on the test animal, that is until after it has produced an infection. A new cycle may then be started in a fly which had escaped on the previous occasion. This point is, I may mention in passing, another argument in favour of the failure of the trypanosomes to establish themselves in the fly being due rather to the flagellates than to any absolute inhibiting quality or condition in the recalcitrant glossina.

Monkey 199 illustrates some particularly important points in regard to the cycle of *T. gambiense* as a whole. It has been shown, by many experiments carried out on Mpumu, that infected buck produce a high percentage of plus flies, but that monkeys infected by means of these flies give in turn only the usual low percentage characteristic of cycles started from monkeys.

The important features are--

1. The long period during which the trypanosomes had been in buck, namely, 15 months.
2. The infection of 199 by direct injection of the blood from the bush-buck.
3. The large percentage of infective flies yielded by 199 when infective.
4. The loss of this last character when the strain is transmitted by flies to other clean monkeys.

Now, the result of a great deal of biological work during recent years has been to establish the general idea that the function of conjugation or nuclear fusion is not reproduction, but the preservation of the characters of the species as a whole, and the neutralisation of the undue tendency to variation produced by

unchecked individual multiplication. In the case of trypanosomes, the individuals run through a relatively very large number of generations in the vertebrate, and in consequence, as is well known, are capable of developing very well-marked strains, which might almost be termed varieties. The function of the fly, as is obvious from these experiments, is to sift out these variations of the individual strains, and to produce a fairly even type. There is at present no sound evidence of conjugation in any trypanosome life-cycle so far worked out, and the question must be left unprejudiced. It is a very plausible suggestion that the great and undoubtedly stimulating change of environment that occurs in the alternation of hosts has gradually led to the suppression, and finally taken the place, of conjugation. This hypothesis would explain the labile characters and the extraordinary merging of species in the trypanosome group, but it is obviously open to much criticism on the score of its speculative nature. Further, such a conception stands only so long as no sound evidence of conjugation in any trypanosome cycle is to hand.

In any case it seems clear that the cycle in the fly as a whole, whether conjugation actually occurs or not, has much of the biological significance of that process. This conception is of some importance to workers dealing with laboratory strains passed directly for long periods without reference to the intermediate host.

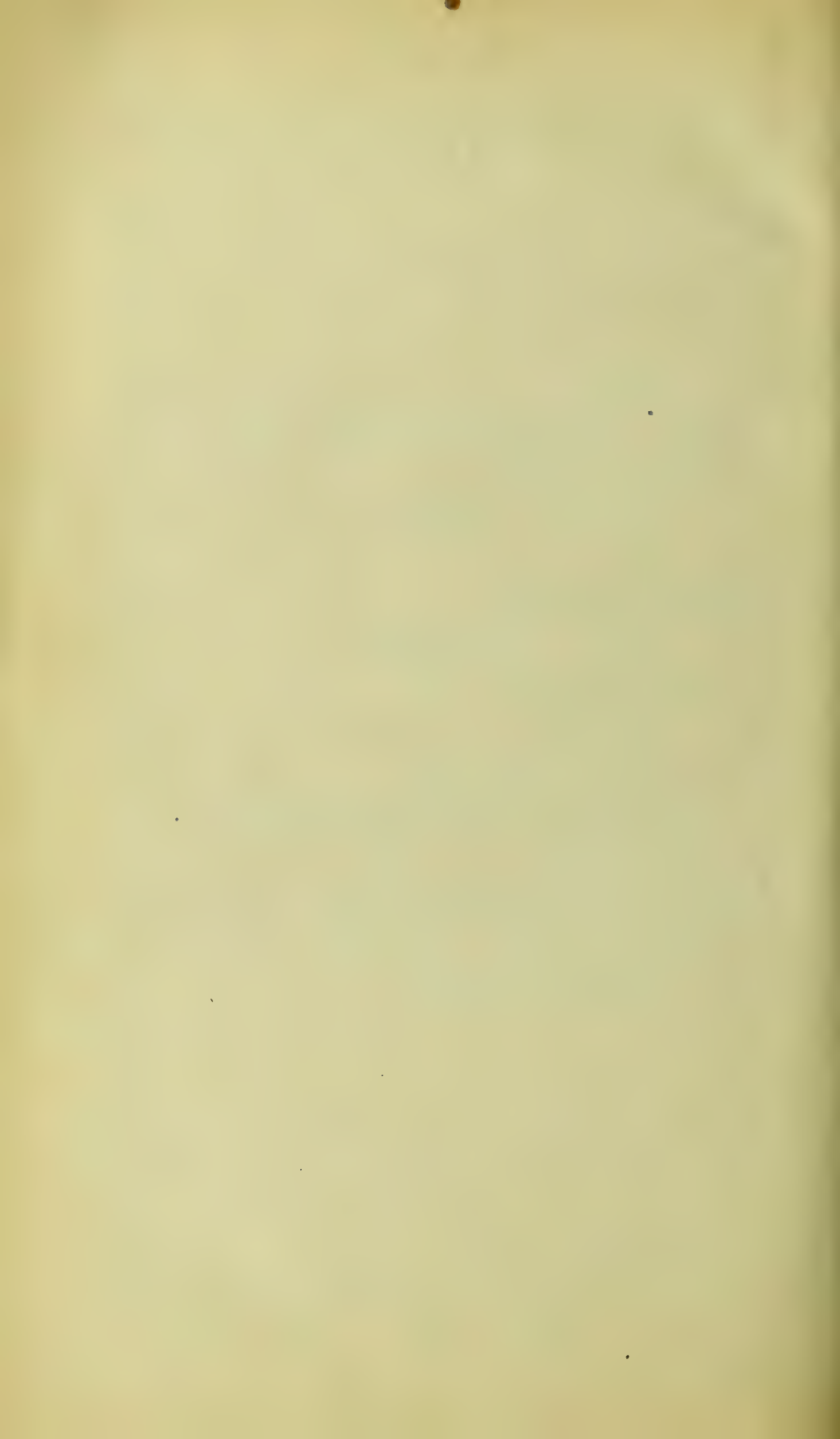
It might be suggested that the fluctuation in the infectivity of the blood of the vertebrate, on which these experiments have laid particular stress, is due to the coming and going of sexual forms in the peripheral blood. The microscopic appearances in the vertebrate have not as yet been sufficiently closely correlated with the infected periods to permit of any statement as to whether there is a morphological distinction in the individuals at different times.

This paper does not deal with the morphological aspect, but I should like to deprecate the rash use of the terms male and female to describe slender and broad trypanosomes. In *T. gambiense* cycles, in the glossina, very slender elongated forms are produced from the broad forms, as in most other trypanosome life-histories; they are naturally present together in the posterior part of the gut, but the former are destined to pass forwards and form the overwhelming majority of the individuals in the proventriculus and thoracic intestine. The terms male and female are gratuitously misleading until the individuals have been shown to have a sexual connection.

Such a use of language encourages an undue bias in the mind of the observer, and, while I am far from denying the possible and even probable occurrence of a sexual process, it is obviously an unscientific procedure to select the individuals *a priori* by the casual method of analogy, an analogy, moreover, not even drawn from the same class of the protozoa.

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I.—ANTELOPE AS A RESERVOIR FOR *TRYPANOSOMA GAMBIENSE*.*

By H. LYNDBURST DUKE.

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In considering the problem of the prolonged infectivity of *Glossina palpalis* along the shore of Lake Victoria Nyanza, the subject must be approached from three points of view:—

Firstly. Some idea of the percentage of infected wild flies must be obtained from time to time, in order to gauge the efficacy of the reservoir.

Secondly. The wild animals frequenting the lake shore must be examined for *T. gambiense*.

Thirdly. The effect of *T. gambiense* on these animals should be studied in the laboratory.

With regard to the requirements (1) and (2), it has been pointed out in a recent report covering the period from March, 1911, to January, 1912, that the percentage of infected flies along the Chagwe coast line is still 0.014 per cent., *i.e.*, a very slight diminution from that reported in June, 1909. During the interim no natives have been allowed in the neighbourhood of the fly, except those in the employ of the laboratory, who are under constant observation.

On Damba Island in May, 1911, Carpenter obtained an infection of a monkey with *T. gambiense*, using 880 wild fly, and two out of four situtunga antelope shot on this island were found to harbour this trypanosome. For Damba Island, therefore, it would appear that the rôle of the antelope in keeping up the infectivity of the wild fly is conclusively established. The conditions on this island are, however, in many ways exceptional, as the fly and antelope are brought into very frequent contact.

Under such conditions it would not be necessary for the antelope to remain infected over a very protracted period of time in order to keep up the infectivity of the fly. In other words, a single infected antelope let loose on Damba Island would soon infect a large number of Glossinæ, which would in turn have access to numerous situtunga. Nothing short of a very rapidly acquired immunity to the disease in the antelope could prevent a relatively large number of fly being infective at any given time.

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On the mainland the opportunities for the fly to bite these animals probably occur much less frequently; and bearing in mind the small percentage of flies capable of carrying *T. gambiense*, it becomes of great importance to know how long an antelope can remain infective.

That these animals may remain infective to fly for a period of at least 10 months after their original infection with *T. gambiense* has already been proved by the study of the antelope in captivity at Mpumu. The animals during this period remain in excellent health, as may be judged from the fact that two young bushbuck have been born in captivity.

The infectivity of these antelope to laboratory-bred *G. palpalis* gradually diminished, until in March, 1911, it appeared probable that the parasites had disappeared from their blood. Up to this date the evidence of infectivity afforded by the presence of trypanosomes in the laboratory-bred *G. palpalis* fed upon the buck, proved sufficiently regular without resorting to blood injection. This latter method was considered a more searching and final test. The experiments given in this paper represent the continuation of these investigations from March, 1910, to January, 1912. During this period laboratory-bred *G. palpalis* were fed upon the antelope, and their blood was also tested by injection into susceptible animals. The injection test, rendering possible the control of considerable quantities of blood, gradually came to supersede the employment of clean flies. There is no reason to doubt that an antelope infective to fly will also give a positive result upon inoculation of its blood into a susceptible animal, and *vice versa*. The objection to the fly test consists in the small proportion of the laboratory-bred flies used which are capable of harbouring the trypanosome. The expenditure of time and trouble is also obviously far greater where a box of flies is concerned than the mere injection of a few cubic centimetres of blood. In several of the more recent experiments the flies were dissected without ever having fed upon a clean susceptible animal, the presence or absence of flagellates being sufficient evidence as to the infectivity of the antelope.

A few preliminary experiments were undertaken to determine whether the animals had acquired any immunity to *T. gambiense*. These are included below. This question has, however, only been superficially dealt with, as the bushbuck selected as most suitable for the experiment became pregnant.

Reedbuck 2357.

Original infection by laboratory-bred flies containing human strain of *T. gambiense*, April, 1910. Latest evidence, previous to this discussion, of the infectivity of this antelope was obtained in January, 1910, when it proved infective to laboratory-bred *G. palpalis*.

With the exception of Experiment 535, all these experiments were fed upon fowls after their original feeds upon the antelope.

Fly Experiments.

Experiment No.	Period for which flies fed upon 2857.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment	Containing flagellates.			
	1911.							
47	May 9-20 ...	29	25	29	0	55	—	
53	May 12-20 ...	34	31	33	0	52	—	
207	July 18-25 ...	98	87	98	1	48	—	41st day fly. Only gut infected. Tryps. +++
282	August 10-16 ...	91	75	91	0	53	—	
532	November 25-30	64	39	64	0	33	—	Never fed on clean monkey.
535	November 29-December 5.	89	61	89	0	37	—	Fed upon monkey throughout experiment.
	Totals ...	405	318	401	1			

The flagellated fly obtained in Experiment 207 was remarkable in showing negative proventriculus and salivary glands, although dissected on the 41st day of the experiment.

Injection Experiments.

Date.	Experiment No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
14.12.11 ...	569	c.c.	c.c.	—	Monkey.
24.1.12 ...	630	8	6	—	"
10.2.12 ...	649	10		—	"

The citrated blood mentioned in the above, and also in the ensuing injection experiment, consists of an admixture of the freshly drawn blood with a citrate solution consisting of sodium citrate 1 per cent. and sodium chloride 0.8 per cent. The mixture is arranged so that the blood just loses its power of clotting, the proportion being about three parts of blood to two of citrate. This antelope was thus shown to harbour *T. gambiense* 15 months after its original infection. Since that date, *i.e.*, a period of seven months, no positive evidence has been obtained.

Reedbuck 2431.

Original infection by laboratory-bred flies carrying human strain of *T. gambiense*, May, 1910. Latest evidence, previous to this discussion, of the infectivity of this antelope obtained in November, 1910, when it proved infective to laboratory-bred *G. palpalis*.

Fly Experiments.

Experiment No.	Period for which flies fed upon 2431.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
93	1911. May 29-June 5	54	43	54	0	51	—	Fed on monkey throughout the experiment.
217	July 22-29 ...	93	89	93	0	46	—	
314	August 21-26 ...	76	55	75	0	51	—	Never fed upon clean monkey. " "
586	December 26-31	73	42	73	0	31		
590	December 28, 1911-January 1, 1912.	73	51	73	0	34		
	Totals ...	369	280	368	0			

Experiments 93 and 314, 586 and 590, were all nourished on fowl's blood during the first 30 or so days of the experiment. In Experiment 93 one fly was found infected with flagellates; these, however, as will be pointed out on another occasion, were derived from the fowl, which was found infected with *T. gallinarum*.

Injection Experiments.

Date.	Experiment No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
23.1.12 ...	69	c.c. 6		—	Monkey.
16.2.12 ...	656	6		—	"

Since November, 1910, therefore, during a period of 15 months, Reedbuck 2431 has given no evidence of infectivity to *T. gambiense*. Attempts will be made forthwith to reinfect this antelope first with flies and subsequently by direct blood injection. In the event of a negative result the serum reactions will then be investigated.

Reedbuck 2359.

Original infection April, 1910, with human strain of *T. gambiense*. Latest evidence prior to this paper of the infectivity of this animal obtained in December, 1911, when it infected laboratory-bred *G. palpalis*.

Fly Experiments.

Experiment No.	Period for which flies fed upon 2359.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
	1911.							
76	May 19-27 ...	67	63	67	0	53	—	
78	May 20-27 ...	49	42	49	0	53	—	
548	December 6-11...	94	59	94	0	40	—	Fed on monkey throughout experiment.
	Totals ...	210	164	210	0			

Experiments 76 and 78 were nourished on cock's blood throughout the earlier days of the experiment.

Injection Experiments.

Date.	Experiment No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
		c.c.			
16.5.11 ...	62		2½ c.c.	—	Monkey.
16.5.11 ...	63		20m	—	White rat.
15.7.11 ...	199	5		+	Monkey.
24.1.12 ...	631	8		—	"
16.2.12 ...	657	7		—	"

In the case of Experiment 199 the incubation period in the monkey was 18 days. A curious point about this monkey, established by my own experiments and those of Miss Robertson, is the relatively large number of positive flies which have been found in boxes fed upon it. Full confirmation is at present lacking, but it would seem that the prolonged sojourn of *T. gambiense* in the buck had resulted in a strain peculiarly adapted to development in *G. palpalis*.*

It will be seen that this buck still harboured *T. gambiense* 15 months after its original infection. For the last seven months, however, no positive evidence has been forthcoming. Experiments to try and re-infect this antelope will be now undertaken.

Bushbuck 2371.

Original infection April, 1910. Latest evidence of infectivity obtained in December, 1910, when it infected laboratory-bred *G. palpalis*.

* Cf. Bushbuck 2371, *infra*.

Fly experiments.

Experiment No.	Period for which flies fed upon 2371.		Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
			1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
80	1911. May 22-27	...	59	50	57	1	53	+	Monkey died of the disease after 130 days.
220	July 24-29	...	110	75	110	0	48	—	Fed on monkey throughout the experiment.
	Totals	...	169	126	167	1			

Experiment 80 was nourished on a fowl during the earlier days. The positive fly showed a characteristic infection of salivary glands and gut, while the proboscis was negative. It was dissected on the 53rd day of the experiment and infected the monkey on the 50th day.

Injection Experiments.

Date.		Experiment No.	Quantity of blood injected.		Result.	Animal used for injection.
			Pure.	Citrated.		
31.5.11	...	97	c.c.	c.c.	—	Monkey.
29.11.11	...	536	5	3	—	"
23.1.12	...	628	7½		—	"
10.2.12	...	653	6		+	"

From the above experiment it will be seen that this antelope proved infective with *T. gambiense* 22 months after its original infection with laboratory-bred *G. palpalis*. The incubation period in the monkey was 10 days.

Previous to Experiment 653 a period of nine months had elapsed without a positive result being obtained. Considerable caution is therefore necessary in pronouncing these antelope negative. It may be noted here that, as in the case of Monkey 199 inoculated from Reedbuck 2359, the strain of trypanosomes in Monkey 653 appears peculiarly adapted to development in *G. palpalis*. Sufficient time has, however, not yet elapsed to definitely establish this point.

Waterbuck 2378.

Original infection, April, 1910. Latest evidence of infectivity afforded by positive *G. palpalis* in August, 1910.

Fly Experiments.

Experiment No.	Period for which flies fed upon 2378.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
344	1911. August 30–September 2	65	45	64	0	42	—	

These flies were nourished on a cock during the earlier days of the experiment.

Injection Experiments.

Date.	Experiment No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
2.5.11	...	35	c.c.	c.c.	Monkey.
22.7.11	...	216	3	4	
23.1.12	...	627	8	4½	
10.2.12	...	650	8	—	

There is therefore no evidence to show that the waterbuck has been infective to *T. gambiense* during the last 18 months. The last positive experiment occurred four months only after the original infection. On August 10, 11, 12, 1911, this waterbuck was fed upon by positive flies. Experiment Box 22, which was subsequently shown to contain an infected fly, and which had proved infective to a number of animals. Sufficient experiments have not been carried out to justify a decision as to the immunity of this waterbuck. These will be undertaken shortly. The size of the animal and its resentment of handling make the satisfactory application of the fly test very difficult.

There is, however, every reason to believe that the infected fly of Box 22 must have fed at least once upon this waterbuck, and the animal apparently resisted re-infection. For some time past it has been suffering from an injured foot, which has prevented more extensive investigations being carried out.

Duiker 648.

Originally infected, February, 1911. Latest evidence of infectivity, April, 1911.

Fly Experiments.

Experiment No.	Period for which flies fed upon 648.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
16	1911. April 24-29 ...	19	16	18	1	96	+	45th day fly.
22	April 25-29 ...	25	24	25	1	123	+	122nd day fly.
13	August 3-4 ...	46	37	46	0	105	-	Miss Robertson's experiment.
	Totals ...	90	77	89	2			

The salivary glands of the positive fly of Experiment 16 were not observed. The fly of September 22 showed a characteristic infection of gut and salivary glands.

Injection Experiment.

Date.	Experiment No.	Quantity of blood injected.		Result.	Animal used for injection.
		Pure.	Citrated.		
6.2.12 ...	637		c.c. 10½	—	Monkey.

Bushbuck 2328.

Originally infected, April, 1910. Latest evidence of infectivity obtained in August, 1910, when this antelope infected laboratory-bred *G. palpalis* with *T. gambiense*.

From June 3 to 10, 1911, 10 months after last evidence of infectivity, this antelope was fed upon daily by Experiment 22. It was desired to ascertain whether any degree of immunity to *T. gambiense* had been acquired, with a view to further experiments with the serum. Bushbuck 2328 has, with the exception of the first few months after infection, always proved negative. As in the case of Waterbuck 2378, the last evidence of infectivity was obtained some four months after infection, the other bushbuck and reedbuck remaining infective for some considerable time longer. It appeared therefore very probable that the trypanosome had died out in this particular antelope.

Fly Experiments.

Experiment No.	Period for which flies fed upon 2328.		Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
			1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
194	1911.								
	July 12-17	...	76	65	76	0	50	—	
206	July 18-25	...	95	78	95	0	48	—	Fed on monkey throughout the experiment.
	Totals	...	171	143	171	0			

Experiment 194 was fed upon cock's blood during the earlier part of the experiment.

Injection Experiments.

Date.		Experiment No.	Quantity of blood injected.		Result.	Animal used for injection.
			Pure.	Citrated.		
18.4.11	...	8	c.c.	c.c.	—	White rat.
18.4.11	...	9		1½	—	Monkey.
28.6.11	...	176	2½		—	Monkey.
28.6.11	...	177		1	—	White rat.
3.8.11	...	244	5½		—	Monkey.
6.2.11	...	638		11½	—	"

In the above table the line indicates the demarcation between experiments carried out before and after the attempt at re-infection in June, 1911. It will be seen that in no case did infection result. This antelope thus appears to have acquired a certain degree of immunity to *T. gambiense*.

The following Experiment 195 represents an attempt to influence the course of an infection of *T. gambiense* in a monkey by treating the latter with the serum of Bushbuck 2328.

Monkey 195 received injections of the serum of Bushbuck 2328 on four occasions as follows:—

On July 12, 1911, monkey received 15 m. serum subcutaneously.

" 22, " " " 30 " "

On August 2, " " " 2½ c.c. " "

" 15, " " " 2 " "

No symptoms of serum-anaphylaxis were observed.

On August 16 to 17, 1911, the monkey was fed upon by positive Experiment 22. On August 30, *T. gambiense* first appeared in the blood of the monkey, the incubation period being thus 13 to 14 days.

On September 3, 1911, the monkey received $2\frac{1}{2}$ c.c. serum of Bushbuck 2328, without any diminution being observed in the number of trypanosomes.

February, 1912, Monkey 195 is still alive and apparently in good health. The interval, however, is not long enough to warrant any criticism as to the effect of the above treatment on the disease, which would, however, appear to be pursuing quite an ordinary course.

The most striking point about this experiment is the long incubation period of the disease in the monkey. Normally after infection with Box 22, trypanosomes appeared in the monkey's blood after an interval of seven days only. Further experiments with this bushbuck had to be discontinued owing to the animal becoming pregnant.

This bushbuck has apparently successfully resisted the attempt to re-infect it with positive flies in June, 1911. Further investigations are essential before a definite opinion can be pronounced as to its immunity. Experiments will be carried out with a view to re-infection by direct blood injection, and following a negative result, the reaction of the serum will be investigated.

The following experiments were undertaken to ascertain whether the serum of these antelope had acquired any immunising properties.

The animal selected was Reedbuck 2359, which at the time, June, 1911, had shown no evidence of infectivity for some seven months. As may be seen above, this animal was subsequently proved to be infective in July, 1911—one month after the experiments were carried out.

(1) Has the serum of Reedbuck 2359 any trypanolytic action on *T. gambiense*?

Two tubes A and B were prepared, each containing $\frac{1}{3}$ vol. serum and $\frac{2}{3}$ vol. citrated *T. gambiense*-blood. Tube A contained serum of Reedbuck 2359; tube B serum of a bushbuck which had never been infected with *T. gambiense*. The tubes were placed for four hours in incubator at 28° C. The contents were examined at intervals during this time, but no difference was observed in the motility of the trypanosomes, and no agglutination was seen in either tube.

(2) Has serum of Reedbuck 2359 any protective action against *T. gambiense*?

(a) On June 15, 1911, a white rat received 15 m. serum of reedbuck, and on June 16, 1911, 24 hours after, was injected with a small dose of *T. gambiense*. The rat died in 25 days.

(b) In two tubes A and B were placed respectively $\frac{1}{3}$ vol. of serum of reedbuck and $\frac{2}{3}$ vol. of citrated *T. gambiense*-blood, and $\frac{1}{3}$ vol. serum of normal bushbuck and $\frac{2}{3}$ vol. of citrated *T. gambiense*-blood. The tubes were kept at room temperature in the dark. The contents of the tubes were examined at intervals of 3, $5\frac{1}{2}$, and $6\frac{1}{2}$ hours, without any differences being noted; $6\frac{1}{2}$ hours after the commencement of the experiment, 6 m. of each tube-contents was inoculated into white rats C and D. These rats died in 23 and 21 days respectively.

The *T. gambiense* employed in the above experiments was a strain derived from wild Lake-shore flies. It is to be regretted

that no control injection of trypanosomes alone was performed, owing to scarcity of rats and my failure at the time to realise the importance of the point.

It will be noticed that the disease in both cases was extraordinarily rapid. Whether this was due to the trypanosome strain, or to the antelope serum, or to both these factors, cannot at present be decided. The monkey from which the trypanosome was taken was killed two months after the infection. In a subsequent experiment, in which a white rat was injected with another strain of Lake-shore trypanosomes derived from fly, the disease lasted 54 days. The various *T. gambiense* strains at Mpumu all give rise to a fairly protracted disease in rats.

The above facts warrant an enquiry as to the identity of the trypanosomes obtained in monkeys by feeding wild Lake-shore *G. palpalis* upon these animals. The trypanosomes so obtained have always been described by Bruce as *T. gambiense*, though I am unaware of the nature of the evidence adduced in support of this assertion. It is, of course, extremely probably, considering the recent epidemic along the Lake-shore and the nature of local cattle diseases, that these trypanosomes found in wild flies and pathogenic to monkeys are *T. gambiense*. Nevertheless, the question cannot be summarily dismissed, owing to the fact that other species of trypanosomes can produce similar symptoms in these animals. The point of practical importance to be established is whether these trypanosomes are pathogenic to man, and this is obviously very difficult to prove.

In a recent paper I have discussed the differential diagnosis of trypanosomes recovered from the Lake-shore and *T. brucei*. The recent discovery that *T. rhodesiense* is susceptible to the action of human serum is of great interest in this connection, since on theoretical grounds alone a trypanosome which succumbed to human serum *in vitro* and in laboratory animals might well be judged non-pathogenic to man. In the present instance it must be borne in mind that, although these Lake-shore trypanosomes, whether found in wild flies or in wild antelope, may have been originally derived from human beings during the recent epidemic, it is possible that the prolonged sojourn in other hosts may have materially modified the reactions of the original "Uganda" strain.

Apart from its pathogenicity to man, there is little in the behaviour of *T. rhodesiense* to suggest close affinity to *T. gambiense*. Its recently discovered susceptibility to the action of human serum, together with many of its animal reactions, suggest rather resemblances to *T. brucei*. This latter trypanosome has up to the present time been generally considered to be non-pathogenic to man. The mere fact, however, that *T. rhodesiense* was originally recovered from man in sufficient to account for the controversy which has arisen over the attempt to separate this trypanosome from *T. gambiense*.

In the same way with these Lake-shore trypanosomes. Whatever be the scale of virulence established, the final diagnosis will always depend upon the behaviour of the organism in the human subject.

In reviewing the above experiments it will be seen that these antelope are apparently very slowly losing their infectivity for *T. gambiense*. The animals are all in excellent health, and in two out of four cases the most recent evidence has been afforded by positive laboratory-bred *G. palpalis*. It would thus seem that the adaptation of *T. gambiense* in these antelope approaches very nearly the biological ideal, *i.e.*, a compromise between the trypanosome and its host, whereby, without injuring the latter, the parasite becomes peculiarly well equipped to survive the insect carrier.

Time alone can show whether this balance between parasite and antelope host can be maintained indefinitely. Judging from the case of Buchbuck 2371 it is quite reasonable to expect evidence of infectivity to crop up occasionally at long intervals in these laboratory animals. Surrounded by wild flies in nature such an antelope as 2371 might well prove a far greater danger than would appear from the long intervals occurring between positive experiments in the laboratory. It is, of course, possible that the trypanosomes may be slowly dying out in the buck, and that a greater or less immunity may be left behind. But even if this be so, an animal which can remain infective for 22 months may be looked upon as a true reservoir for the virus of sleeping-sickness. It has only to remain in the neighbourhood of the fly to perpetuate the disease indefinitely among susceptible Lake-shore animals.

The behaviour of the positive flies obtained from Antelope 2371 and 2357, 13 and 15 months respectively after the original infection, is interesting. In the first case, the positive fly did not infect the monkey until the 50th day of the experiment; and in the second instance, a 41-day fly showed no flagellates in either proventriculus or salivary glands. In all previous experiments with these antelope the flies became infective about the 30th day of the experiment.

An explanation of this curiously slow development of the flagellates in the fly may be that the trypanosomes are present in very small numbers in the peripheral blood of the buck. A fly would then obtain an extremely small number of trypanosomes at a feed, whereas formerly a considerable number may have been imbibed.

Some support is lent to this suggestion by the prolonged incubation period in Experiment 199 (Reedbuck 2359), where 18 days elapsed before *T. gambiense* appeared in the monkey's blood.

Conclusions.

1. That the antelope may remain capable of infecting *G. palpalis* with *T. gambiense* for a period of at least 22 months after their original infection with this trypanosome.
 2. That there is some evidence to show that an antelope which has ceased to be infective for *T. gambiense* acquires some degree of immunity against re-infection.
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2.—NOTES ON *TRYPANOSOMA GAMBIENSE* AND *GLOSSINA PALPALIS*.

By H. LYNDBURST DUKE.

(Received 11 May, 1912.)

A.—SOME OBSERVATIONS ON THE PHYSIOLOGY OF *T. GAMBIENSE* IN *G. PALPALIS*.

The following experiments were undertaken to ascertain whether the nature of the blood ingested by the flies during the earlier days of an experiment had any effect on the development of *T. gambiense* in the fly. With this object a series of paired boxes was taken and fed for the same period upon a monkey whose blood contained *T. gambiense*. Throughout the experiment both boxes received parallel treatment as regards the alternation of feedings. One thus served as a control to the other.

The monkey selected for the infecting feeds was that of Experiment 199, which was infected by direct inoculation from a reed-buck 15 months after the original infection of that animal. This strain of trypanosomes in the Monkey 199 has proved exceptionally well adapted to development in *G. palpalis*.

Experiment 358.

Date.	Day of the Expt.	Procedure.	Result.	Remarks
1911.				
Sept. 3 ...	1	Fed on monkey 199.		
" 4 ...	2	Starved.		
" 5-25 ...	3-23	Fed on bushbuck 123	—	6 positive flies dissected; gut containing trypanosomes. No infected proventriculus or salivary glands found.
" 26 ...	24	Starved.		1 positive fly died. Flagellates in gut and thoracic gut. Proventriculus and salivary gland, nil.
" 27-Oct. 7 ...	25-35	Fed on monkey 390	+	76 flies alive on 30th day.

At the final dissection of this experiment six more positive flies were obtained, four of which showed virulent salivary gland infections. This gives a total of 13 flagellated flies out of a total of 113 flies dissected during the experiment, *i.e.*, 11.5 per cent.

Experiment 357 (control to 358).

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1911.				
Sept. 2-3 ...	1	Fed on monkey 199.		
" 4 ...	2	Starved.		
" 5-25 ...	3-23	Fed on fowl 364.		1 positive gut + + + only.
" 26 ...	24	Starved.		
" 27-Oct. 6 ...	25-34	Fed on monkey 391	+	44 flies alive on 30th day.

At the final dissection of this experiment one positive fly was found with numerous flagellates in the salivary glands. This gives two positive flies in a total of 75 dissected during the experiment, *i.e.*, 2.6 per cent.

Experiment 567.

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1911.				
Dec. 13-15 ...	1-2	Fed on monkey 199.		
" 16 ...	3	Starved.		
1912.				
" 17-Jan. 1 ...	4-19	Fed on bushbuck 123	—	
Jan. 2 ...	20	Starved.		
" 3-17 ...	21-35	Fed on monkey 595	+	1 positive fly died. Flagellates present in gut only. + + +. 28 flies alive on 30th day.

At the final dissection of this experiment one positive fly was found with salivary glands well infected. Thus in a total of 56 flies dissected 2 or 3.5 per cent. contained flagellates.

Experiment 568 (control to 567).

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1911.				
Dec. 13-15 ...	1-2	Fed on monkey 199.		
" 16 ...	3	Starved.		
1912.				
" 17-Jan. 1 ...	4-19	Fed on clean monkey 577.	—	
Jan. 2 ...	20	Starved.		
" 3-20 ...	21-38	Fed on monkey 596	—	19 flies alive on 30th day.

Both monkeys remained negative. No positive flies were obtained throughout the experiment. 43 flies were dissected.

Experiment 581.

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1911. Dec. 21-23 ...	1-2	Fed on monkey 199.		
„ 24-25 ...	3-4	Starved.		
1912. „ 26-Jan. 15	5-25	Fed on sheep 583.	+	10 positive flies died shewing flagellates in gut. Only one shewed Trypanosomes in the proventriculus. Salivary glands not seen.
Jan. 16 ...	26	Starved.		
„ 17-23 ...	27-33	Fed on monkey 615.	+	2 positive flies died. Salivary glands of one + + +, of other not seen.
„ 24-25 ...	34-35	Starved and dissected.		2 positive flies both showing salivary glands + + +. 27 flies alive on 30th day.

Both sheep and monkey became infected with *T. gambiense*, the sheep on about the 25th day of the experiment. Out of 68 flies dissected 14 contained flagellates, *i.e.*, 20·6 per cent.

Experiment 582 (control of 581).

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1911. Dec. 21-23 ...	1-2	Fed on monkey 199.		
„ 24-25 ...	3-4	Starved.		
1912. „ 26-Jan. 24	5-34	Fed on clean monkey 584.	+	1 positive fly died. Gut only infected 79 flies; alive on 30th day.
„ 25-27 ...	35-37	Starved and dissected.		2 positive flies: swarming gut and proventriculus salivary glands not seen.

Monkey 584 became infected on the 29th day of the experiment. Out of 82 flies dissected 3 contained flagellates, *i.e.*, 3·6 per cent.

Experiment 618.

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1912.				
Jan. 17-18 ...	1	Fed on monkey 199.		
" 19 ...	2	Starved.		
" 20-Feb. 8 ...	3-22	Fed on sheep 633 ...	+	
Feb. 9 ...	23	Starved.		
" 10-16 ...	24-30	Fed on monkey 652	+	61 flies alive on 30th day.
" 17-20 ...	31-34	Starved and dissected.		3 positive flies: all salivary glands infected.

Sheep 633 became infected about the 22nd day of the experiment. 81 flies were dissected during the experiment, and of these 3—*i.e.*, 3·7 per cent.—contained flagellates.

Experiment 617 (control of 618).

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1912.				
Jan. 17-18 ...	1	Fed on monkey 199		
" 19 ...	2	Starved ...		
" 20-Feb. 15...	3-29	Fed on monkey 624	—	
Feb. 16-19 ...	30-33	Starved and dissected		59 flies alive on 30th day.

No positive flies were obtained in this experiment. 70 flies were dissected.

Experiment 127.

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1912.				
March 8-9 ...	1	Fed on monkey 653		Monkey 653 shewing Trypanosomes + + + died on March 11.
" 10 ...	2	Starved.		
" 11-21 ...	3-13	Fed on calf ...	—	
" 22 ...	14	Starved and dissected		

Forty flies were dissected during this experiment, none of these contained flagellates.

Experiment 128.

Date.	Day of the Expt.	Procedure.	Result.	Remarks.
1912.				
March 8-9	... 1	Fed on monkey 553.		
" 10	... 2	Starved.		
" 11-23	... 3-15	Fed on clean monkey 631.	—	
" 24-26	... 16-18	Starved and dissected.		

Fifty-nine flies were dissected during the experiment of which 2 contained flagellates.

The above two experiments were conducted by Miss Robertson, who kindly allows me to use them. It will be noticed that the results are not in keeping with those carried out with sheep and bushbuck. On dissection of the two positive flies no flagellates were found in the proventriculus and the number present was comparatively small considering the day of development.

In the other experiment carried out with Monkey 653 flagellates were invariably found in the proventriculus by the 18th day, and in one case an infection was obtained on the 20th day of the experiment. It is thus possible that the approaching death of the monkey had altered the behaviour of this strain of trypanosome in the fly.

The following table is a brief summary of the positive flies in the above experiments.

Expt. No.	Percentage of positive flies with ~~goat~~ or buck blood.	Percentage of positive flies with cock or monkey blood.
	per cent.	per cent.
357 and 358	11·5	2·6
567 " 568	3·5	0
581 " 582	20·6	3·6
617 " 618	3·7	0
127 " 128	0 (calf blood)	3·3

From the above it will be seen that the percentage of positive flies is considerably greater in those experiments which had been fed upon sheep or bushbuck blood.

It must be clearly understood that these experiments were not especially selected as giving support to the criticisms advanced in this discussion. They represent all the investigations carried out on this subject up to the time of writing. In spite of the contradictory result obtained with the calf, the evidence of the first four experiments is sufficiently definite to afford a striking contrast to the results of Kleine and Fischer.* These observers, using, it is true, a far greater number of flies, obtained approximately 10 per

* 'Die Rolle der Säugetiere bei der Verbreitung der Schlafkrankheit' (Zeitschrift für Hygiene und Infektions-Krank. Vol. vii. 1911).

cent. of infected flies when monkeys were used throughout the experiment against 2.4 per cent. when ruminants were employed. The corresponding figures for the above experiments are:—

Of 329 flies nourished on fowl or monkey blood 2.1 per cent. were found infected.

Of 358 flies nourished on fowl or ruminant blood 9.1 per cent. were found infected.

The obvious criticism of these results is that the difference is due to the flies having picked up flagellates other than *T. gambiense* from the sheep or bushbuck employed. This objection is a serious one. In dealing with a positive fly it is impossible to diagnose the flagellates seen unless some gross specific character is present, such as marked crithidial form or the occurrence of flagellates in the proboscis tube. The presence of the trypaniform flagellates in the gut alone is a stage common to several different mammalian trypanosomes. The following control observations were accordingly made to endeavour to prove that the sheep and bushbuck of the above experiments were free from sources of the error. It must, however, be admitted that in the case of the sheep the evidence is hardly final, although the probabilities are all against a fallacy.

Bushbuck 123.

This antelope was infected with *T. uniforme* at the laboratory in January, 1911. For the last twelve months it has shown no evidence of infectivity. It will be remembered that *T. uniforme*, although very readily transmitted by *G. palpalis*, is confined exclusively to the proboscis of the fly, and has never been observed in the gut.

T. uniforme, therefore, even if still present in the bushbuck, cannot be considered as a fallacy in the above experiments.

From July 20 to August 8, 1911, 67 clean laboratory-bred *G. palpalis* were fed upon this bushbuck every other day. Subsequently they were fed upon a clean monkey until September 4. On September 5 the flies were starved and dissected, and no infected flies were found. 63 flies were alive on the 30th day.

From October 2, 1911, 49 clean laboratory-bred *G. palpalis* were fed upon this bushbuck for 5 days. Subsequently they were fed upon a fowl for 27 days. During the experiment 47 flies were dissected and no flagellates were seen either in the gut or proboscis. 37 flies were alive on the 30th day of the experiment.

From December 17, 1911, 66 clean flies, laboratory-bred, were fed upon the bushbuck for 14 days, and subsequently fed upon a fowl. On the 30th day 55 flies were alive. Throughout the experiment 66 flies were dissected and no flagellates found in gut or proboscis.

In these three experiments, therefore, a total of 180 flies were dissected with negative results; 155 of these were alive on the 30th day.

On January 22, 1912, 6½ c.c. citrated blood of Bushbuck 123 were injected subcutaneously into a clean monkey. The monkey remained healthy.

On February 6, 1912, 4½ c.c. citrated blood of Bushbuck 123 were injected into a clean monkey, also with a negative result.

Sheep 583.

This animal had never been infected with trypanosomes at the laboratory. Between September 15 and December 23, 1911, the blood of this animal was examined on 57 occasions with negative results.

Sheep 633.

Infected at the laboratory with *T. uniforme* early in March, 1911.

Since May, 1911, has shown no sign of trypanosomiasis, although examined from time to time.

From January 18, 1912, was examined on 18 occasions with negative results, until, on February 16, *T. gambiense* appeared in its blood, confirmed by the subinoculation into a monkey.

The above results are of especial interest in view of a paper recently published by Kleine and Fischer from Tanganyika.¹ These authors arrive at totally different conclusions to those reached by Uganda workers. They conclude that man and monkeys are far more important as a reservoir for sleeping sickness than the local ruminants. The whole trend of the work done at Mpumu would point to the opposite conclusion, and the above experiments are in keeping with this assertion. Kleine's observations that a fly which has proved infective to monkeys may fail to infect a sheep and goats² has, so far as I am aware, no parallel in the reports from Uganda. Once a fly has proved infective for *T. gambiense* experience points to that fly remaining infective to all animals susceptible to *T. gambiense* until its death. In this way, before the point was under dispute, a situtunga and a duiker were infected at this laboratory by flies which had been nourished on cock and monkey blood. This also almost certainly applies to those flies carrying a human strain of *T. gambiense* with which the 1908 to 1910 Commission originally infected the numerous antelope at present at Mpumu.

From unpublished results of Miss Robertson which she kindly allows me to quote, it would seem that *T. gambiense* must become established in the salivary glands before a fly becomes infective. This was also the opinion of Bruce and his collaborators. The function of the fly in relation to *T. gambiense* is presumably the production of the salivary-gland type of flagellate, which—again presumably—is capable of infecting all animals susceptible to *T. gambiense*. Kleine's results impose an obligation on the trypanosome in the fly which compels it to discriminate between two susceptible animals, a goat and a monkey.

The conditions are doubtless very different at Tanganyika and Mpumu both as regards the strain of trypanosome and the climate. This must account largely for the discrepancies between the two sets of experiments. Nevertheless, on studying the above-mentioned paper two criticisms are at once suggested.

(1) 'Die Rolle der Säugetiere bei der Verbreitung der Schlafkrankheit' (Zeitschrift für Hygiene und Infectious-Krankheiten. Vol. vii. 1911).

(2) Cf. *ibid.* Table IV., note (1).

1. The verification of the negative microscopical diagnosis of the experimental sheep and goats by subinoculation into monkeys. This, of course, implies a plentiful supply of these animals.

2. In quoting the results of fly-dissection no reference is made to the presence or absence of flagellates in the salivary glands. It is thus possible that some of Kleine's "infizierte Fliegen" showed no salivary-gland infection—in other words, were incapable of infecting.

There is yet another point which apparently receives no attention, and which is also being established in the course of Miss Robertson's work. This is the occurrence of periods in the subject of trypanosomiasis when, quite irrespective of the mere presence of flagellates in the peripheral blood, the trypanosomes appear incapable of full development in the fly. For this information I am again indebted to Miss Robertson. This factor is at least as important in determining a negative experiment as is the nature of the nutriment material supplied to the trypanosomes in the fly.

B.—TO STUDY THE EFFECT OF DIFFERENT KINDS OF BLOOD ON THE LONGEVITY OF LABORATORY-BRED *G. palpalis*.

In the following table a series of experiments are given in which, after a varying number of initial feeds, laboratory-bred flies are left without further feeding until death. Various animals were selected for the original feeds. The cages were subsequently left under similar conditions of temperature and humidity.

Exp. No.	Total flies used.		Originally fed upon.	Death Roll. Days after last feed.																		
	♂	♀		1-10 ♂ ♀	11	12	13	14	15	16	17	18	19	20	21							
257	35	21	3 days on fowl	{ 1	5	5	5	6	3	3	5	2										
258	28	29	3 " normal monkey	{ 0	11	3	4	3	4	7	0	0	1	3	4	2	0					
320	31	31	4 " duiker	{ 14	6	5	2	2	2	2	0											
321	27	48	4 " goat	{ 21	5	1	2	1	0													
				{ 18	5		3	2	1	0												
				{ 40	4	3	1	4	3	2	0											
254	25	25	3 " reed buck ..	{ 3	0	11	4	3	3	4	5	2	0									
				{ 1	4	4	4	4	3	2	0											
365	19	11	3 " normal monkey	{ 2	2	6	1	0	0													
				{ 2	2	2	2	3	0													
366	9	8	3 " arsenic	{ 4	3	1	0															
				{ 0	9	3	5	1														
367	19	11	3 " reed buck ..	{ 3	3	2	3	0	1	0												
				{ 2	2	1																
439	26	24	{ 2 days on normal monkey	{ 23	0	1																
			{ 2 days starve																			
			{ 1 day on normal monkey																			
			{ 2 days on bush buck ..																			
457	23	27	{ 2 days starve	{ 15	6	2	0	0	0	0	1											
			{ 1 day on bush buck ..	{ 22	3	1																

The weak point in the above experiments are the impossibility of obtaining a number of flies of exactly the same age, and also the difficulty of ensuring satisfactory feeding by all the flies in an experiment.

C.—TO PROVE THAT *Glossina palpalis* INFECTIVE FOR *T. gambiense* IS CAPABLE OF INFECTING MONKEYS ON THREE CONSECUTIVE DAYS.

Positive fly Experiment 22 was fed on July 26, 1911, on Monkey 200; on July 27, 1911, it was fed on Monkey 201; and on

July 28, 1911, on Monkey 202. Monkeys 200, 201, and 202 showed *T. gambiense* in their blood after 7, 9, and 9 days' incubation period respectively.

On dissection of Experiment 22 only one positive fly was found which must have infected all these monkeys. This fly showed numerous flagellates in gut and salivary glands.

D.—SHOWING THAT THE FEEDING OF A LARGE NUMBER OF NEGATIVE *Glossina palpalis* ON A MONKEY DOES NOT INTERFERE WITH SUBSEQUENT INFECTION OF THE MONKEY WITH POSITIVE FLIES.

During February and March, 1911, Monkey Experiment 641 was fed upon by some 8,000 wild Kibanga *Glossina palpalis*. In November, 1911, this monkey was fed by positive fly Experiment 74, and became infected in the ordinary way with *T. gambiense*.

Mpumu,

April 9, 1912.

3.—OBSERVATIONS ON FOWLS AND DUCKS IN UGANDA WITH RELATION TO *TRYPANOSOMA GALLINARUM* AND *T. GAMBIENSE*.*

By H. LYNTHURST DUKE. (With a Note by Miss MURIEL ROBERTSON.)

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S.
Received May 9,—Read June 6, 1912.)

[Plate 1.]

In the course of a considerable number of experiments, carried out to investigate the duration of infectivity of antelopes for *T. gambiense*, Uganda fowls were employed to nourish the flies during the earlier days of the experiments. The birds have been shown by the Commission of 1908-10 to harbour *T. gallinarum* in a certain number of instances, although nothing was known concerning the intermediate host of this trypanosome.

In the course of routine dissections of laboratory-bred flies from these experiments a curious crithidial type of flagellate was noticed in the hinder part of the mid-gut, similar to that reported recently from this laboratory by Captain A. D. Fraser and myself. These flagellates must have been derived either from the antelope upon which the flies originally fed or from the cock. In morphology and movement they were distinguishable at once from developmental stages of *T. gambiense* in *Glossina palpalis*. As some of the laboratory antelope had been found to be infected with *T. ingens* it appeared possible that the flagellates might represent developmental stages of this trypanosome. On the

* Reprinted from Proceedings of the Royal Society, Ser. B, vol. 85, p. 378.

Table I.

Expt. No.	Number of flies fed upon cock.	Expt. No. of cock.	Examination of cock's blood for <i>T. gallinarum</i> .	Flies showing crithidiæ.	Day after first feed on cock.	Number of flies.	
						1st day.	30th day.
158	17	110	+	0	18	56	52
227	21	210	+	1		87	70
214	27	110	+	0		55	52
Totals ...				1		198	174

Remarks.—The flagellates were present in the hinder part of the mid-gut only, and in large numbers.

Table II.

Expt. No.	Number of days fed on cock.	Expt. No. of cock.	Examination of cock's blood for <i>T. gallinarum</i> .	Flies showing crithidiæ.	Day after first feed on cock.	Number of flies.			Cage originally fed upon.
						1st day.	30th day.	Dissected.	
93	12	110	+	1	14	54	43	54	Reedbuck 2,431.
383	26	384	+	1	54	112	98	112	Monkey 199 (<i>T. gamb.</i>).
394	17	431	—	3	14, 35, 49	91	66	88	" 392 (arsenic).
428	12	462	—	1	4	119	66	118	" 199 (<i>T. gamb.</i>).
429	11	465	—	1	19	89	63	89	" 199
453	17	460	+	1	27	101	75	100	" 401
Totals ...				8		566	411	561	

Remarks.—The positive fly of Experiment 453 showed an infection of the proventriculus. In all other infected flies, flagellates were only present in the hinder region of the mid-gut.

other hand suspicion fell upon *T. gallinarum*, although at the time microscopical examination of the fowls used had always proved negative.

To solve the point clean laboratory-bred flies were fed upon the suspected cocks, with the result that after two failures the crithidiæ were obtained in the fly. The connection between *T. gallinarum* and these crithidiæ was further established on several occasions by the subsequent discovery of the trypanosomes on repeated examination of the cock's blood. Owing to the extreme difficulty of making certain that any given fowl was not infected with this trypanosome, it was found practicable to conduct any systematic experiments on its development in *G. palpalis*. The actual day of development of any given infection cannot be established, as in every case the flies were a considerable time on the infected cock.

Although the blood of all the fowls employed was examined on numerous occasions, *T. gallinarum* was not always to be found in those birds which were proved by fly-dissection to be infected. The trypanosomes are very rare in the peripheral circulation, and even on centrifugalisation of considerable quantities of blood after death were only obtained in very small numbers.

The preceding tables show the cycle and other experiments in which these crithidiæ were seen in the course of the fly-dissection. In Table I. the flies were fed upon infected cocks only. In Table II. the flagellates were found incidentally in the course of cycle-experiments with *T. gambiense*.

The following Description of the Morphology of the Flagellates has been most kindly drawn up for me by Miss Robertson, to whom all positive flies were handed over:—

“ In many respects the appearance of *T. gallinarum* in *G. palpalis* is exceedingly characteristic. The infection is rarely very numerous, seldom assuming anything like the virulence of *T. gambiense* or *T. nanum* in the same situation.

“ The trypanosomes are, as a rule, situated pretty far back in the alimentary tract. The mid-gut of the fly, that is to say, the region between the proventriculus and the origin of the Malpighian tubules may conveniently be divided into three portions, anterior, middle, and posterior. The infection very rarely proceeds further forward than the middle portion. On one occasion only were these trypanosomes found in the anterior portion and in the proventriculus.

“ The motion of the flagellate in the live state is very striking, and at once arrests the attention of any observer accustomed to the appearance of gut-forms of mammalian trypanosomes. The body is in the vast majority of the forms quite stiff through about two-thirds of its length, and is often broad and relatively massive, though the breadth is subject to variation. The flagellum is much thicker than in mammalian trypanosomes, and has a lashing motion, which drags the organism forward with a rapid movement

of translation. This progression takes place in straight lines, and in the more slender drawn-out individuals may occasionally be reversed in direction.

"As it is impossible to tell when the flies first received the infecting trypanosomes, I have no information as to the exact sequence of forms in the cycle. The main outlines are, nevertheless, pretty obvious, and the massive broad forms gradually give place to smaller and more slender crithidial individuals, some of which, in what appears to be the later flies, show a considerable lengthening out of the body.

"As will be seen from the drawings (Plate 1), true trypanosome-phases have not been observed. Multiple division takes place, and is not by any means a very rare occurrence, although here, as in most trypanosome-cycles, binary fission is the usual method of multiplication (*see* Figs. 1, 2, 4, and 9). The process in question is rather irregular, and, after carefully studying a considerable number of these appearances, it becomes clear that it is a mere telescoping of successive binary divisions.

"The flagellate contains in the earlier infections a relatively large amount of dense protoplasm, and we have thus an illustration of the common biological process, whereby, in a cell encumbered with much protoplasm, the nuclear apparatus may undergo several successive divisions before the correlated splitting of the cell-body is accomplished.

"*Morphology.*—The films were fixed wet, by dropping them face downwards on to Schaudinn's fluid. The stains used were iron-haematoxylin, after Heidenhain's method, and hæmalum.

"The protoplasm, especially in the broader forms, is very dense and granular, a feature which is somewhat less marked in the later stages. Unfortunately, this density of the protoplasm obscures the final cytological details to a greater or less extent.

"The trophonucleus is of a common trypanosome type, consisting of a large central karyosome surrounded by a clear space, which is bounded in turn by a delicate membrane; fine rays pass from the karyosome to the membrane.

"The kinetonucleus (centrosome of French and blepharoplast of German writers) lies close to and generally in front of the trophonucleus. It is relatively large, and presents the somewhat curious double appearance of two closely-opposed granules lying one behind the other. This does not seem to be in any way a case of precocious division.

"In Fig. 4, where division is actually in process, and where the two kinetonuclei are still joined by the centrodosome, they nevertheless preserve the double appearance, and, in a case of multiple fission figured (Fig. 9), the same thing is to be observed. Moreover, the direction of the plane of division is at right angles to the split in the kinetonucleus. In some of the latest stages of the material, namely, those from the proventriculus, this is less apparent, and the kinetonucleus seems more compact; still, even here the double nature of the structure can still be detected in most cases.

“ The blepharoplast proper, *i.e.*, the minute granule actually at the origin of the flagellum, is usually obscured, but can be seen in favourable specimens.

“ Another structure not usually to be observed in trypanosomes, but which I have seen in the development stages of *T. raia*, a massive trypanosome not unlike *T. gallinarum* in appearance, is a granule situated towards the posterior, *i.e.*, antflagellar, end of the cell. This granule is not particularly small in size, but its outline is rarely very sharply defined; in some instances it appears to be attached by a fine line to the nucleus or to the blepharoplast. In some individuals there are two lines visible, one of which passes to the blepharoplast, the other to the nucleus. Owing to the density of the protoplasm these lines are not very clearly visible, and can be observed only in the more favourable specimens.

“ The granule, on the other hand, is a very fairly constant appearance, and divides at divisions (Figs. 1, 2, 4, and 9). In *T. raia*, in the developmental stages in the leech, these appearances are much clearer and more perfectly defined. In *T. gallinarum* the granule is not to be seen in the more slender stages.

“ Figs. 1, 2, and 4 give an illustration of the more usual binary fission, the spindle of the trophonucleus shows the characteristic centrodesmose, but the finer details are never particularly clear.

“ In conclusion, I may say that, while I have not so far met with a mixed infection, it appears that there would not be much likelihood of these forms passing undetected; nevertheless, crithidial individuals appearing in a cycle of a mammalian trypanosome, where cocks' blood had formed part of the food of the fly, would have to be rejected until confirmed from flies fed exclusively upon clean animals.

“ *Note.*—From the nature of the above infections, it appears that *Glossina palpalis* is certainly not the normal host of this trypanosome, and probably not even a facultative host. It is interesting to note in this connection that a small Simulid fly (species so far unidentified) was found relatively frequently on the fowls in certain parts of Bugerere. On one occasion at Endeba, where the fly was more especially numerous, one specimen was found well infected with a trypaniform flagellate. The fowls in the enclosure were found to be infected with *T. gallinarum*. The flagellates in the Simulid were of small size and mostly crithidial in type, though a few showed the nuclear relations characteristic of trypanosomes.

“ No connection between the fowl-parasite and that of the Simulid has been as yet established, but from the habits of the fly it is highly probable that it might play some part in the transmission of the trypanosome in question.—MURIEL ROBERTSON.”

Are Ducks capable of Acting as a Reservoir of T. gambiense?

These experiments were undertaken on the same lines as those carried out by the 1908 to 1910 Commission with the Uganda fowl.

It seems possible that ducks might react differently to *T. gambiense*, and throw some light on the part played by the enormous numbers of aquatic birds along the shores of Victoria Nyanza in relation to sleeping sickness.

Two Muscovite ducks were obtained from Mr. Walsh's farm, as wild birds were not procurable alive. The method of procedure followed consisted in first feeding laboratory-bred flies upon the ducks to ascertain whether the latter harboured any natural infection. The birds were then fed upon by infected flies and afterwards again tested with clean flies, and finally by blood inoculation into a susceptible animal. The blood was also examined from time to time microscopically.

Are the newly arrived Ducks capable of Infecting Laboratory-bred Flies with Flagellates.

Experiment 153.—From June 16 to 20, 1911, 45 clean laboratory-bred flies were fed upon the two ducks, two days on each bird. The flies were then fed for 27 days on a clean monkey and finally dissected. No flagellated flies were found.

Table. III.

Expt. No	Period for which flies fed upon Duck 144.	Number of flies.				Length of experiment in days.	Result.
		1st day.	30th day.	Dissected.	Containing flagellates.		
193	1911. July 10-17	64*	54	64	0	50	—
236	Aug. 1-3 ...	81*	63	81	0	36	—
299	Aug. 16-19	89*	82	89	0	42	—
594	1912. Jan. 1-16 ...	111*	53	111	0	32	—
	Totals ...	345	252	345	0		

* Fed upon clean monkey throughout the experiment.

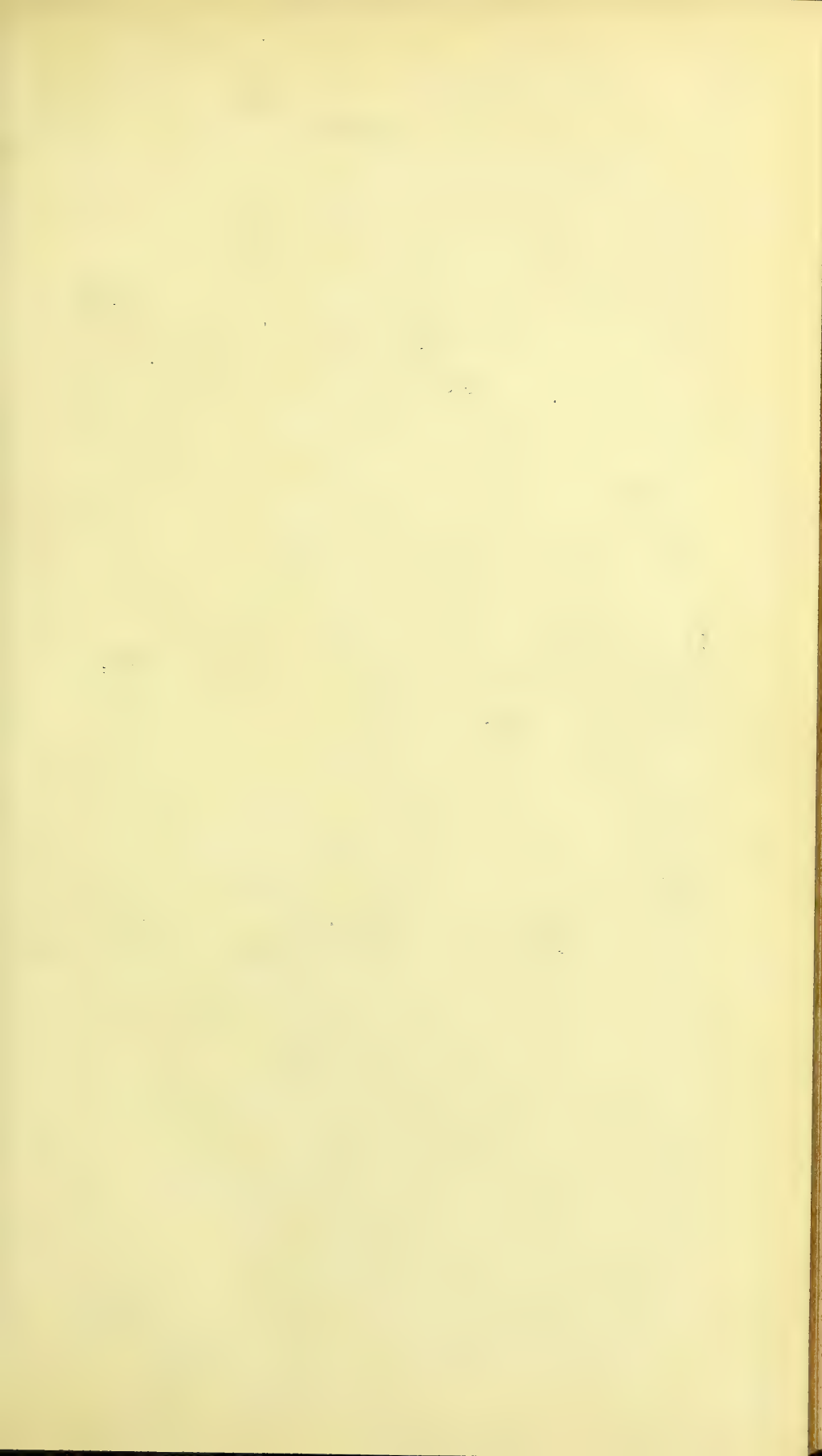
Remarks.—All these experiments proved negative, no flagellates being found in the dissected flies.

From June 19 to 24, 1911, duck Experiment 144 was fed upon daily by flies known to be infective for *T. gambiense*. After a lapse of 16 days laboratory-bred flies were fed upon Duck 144, as shown in the preceding table.

On January 16, 1912, Duck 144 was killed and 6 c.c. of its citrated blood injected into a monkey. This monkey remained healthy and never showed trypanosomes in its blood.

Conclusions.

1. That *T. gallinarum* can multiply in the gut of *G. palpalis*, although this fly is probably not the normal host.
2. That Muscovite ducks are not capable of acting as a reservoir for *T. gambiense*.





DESCRIPTION OF PLATE.

The figures were all drawn at a multiplication of approximately 3,000 diameters with the aid of the drawing apparatus of Abbé.

Fig. 1.—Division stage showing the double appearance of the kinetonucleus and the two trophonuclei.

Fig. 2.—Division stage showing centrodesmose of the trophonucleus spindle.

Fig. 3.—Slender long form.

Fig. 4.—Division stage showing centrodesmose of the kinetonucleus division apparatus; not double appearance of the kinetonuclei.

Fig. 5.—Short, broad form from proventriculus.

Figs. 6 and 7.—Other forms from proventriculus.

Fig. 8.—Typical broad form showing posterior granule and lines to trophonucleus and kinetonucleus.

Fig. 9.—Multiple division.

Figs. 10 and 11.—Very typical forms from the ordinary type of gut infection.

Figs. 12 and 13.—Slender forms from proventriculus; these forms are also found further back in the mid and hind gut.

4.—FURTHER OBSERVATIONS ON THE RECOVERY OF
TRYPANOSOMA GAMBIENSE FROM *TRAGELAPHUS*
SPEKEI ON THE ISLANDS OF LAKE VICTORIA
NYANZA.*

By Dr. H. LYNDBURST DUKE.

(Communicated by Sir John Rose Bradford, K.C.M.G., Sec. R.S.
Received July 17, 1912.)

In a paper† dealing with the recovery of a trypanosome from wild antelope on Damba Island, Lake Victoria Nyanza, the diagnosis is discussed at some length with a view to the exclusion of *T. brucei*.

Fifteen subinoculation experiments were considered and the conclusion was arrived at that the available evidence pointed to the organism being *T. gambiense*. After a lapse of some four months this opinion has received considerable support from a more prolonged investigation of the animal reactions of the trypanosome. In the following table many of the original subinoculations are followed to their conclusion on the death of the animal, and several other experiments have been added to the list.

It will be seen that the evidence supplied by the rat experiments is strongly in favour of *T. gambiense*.

In the six experiments conducted with rats the average duration of the disease is 61 days. This constitutes very strong evidence

* Reprinted from the Proceedings of the Royal Society, Ser. B, vol. 85, p. 483.

† "Antelope and their Relation to Trypanosomiasis," Roy. Soc. Proc., B, vol. 85, p. 156.

against *T. brucei*. In guinea-pigs (three experiments) the average duration is 67 days: in five completed monkey experiments, 147 days.

Animal.	Expt. No.	Source of virus.	Incubation in days.	Duration of disease in days.	Remarks.
Monkey ...	401	Situtunga	8	144	
		402-3.			
" ...	511	" 509-10	12-13	144	
" ...	504	Flies from 401	?	195	
" ...	525	Goat 512 ...	10	207	
" ...	543	Calf 478 ...	10	...	Still alive after 192 days. Active; shows no marked symptoms. Slight wasting.
" ...	575	Goat 579 ...	6	46	This monkey was an old one, and somewhat emaciated at commencement of experiment.
White rat	477	Monkey 401 ...	5	83	
" "	571	" 401 ...	5	44	A young rat.
" "	639	" 511 ...	7	51	
" "	640	" 511 ...	7	52	
" "	646	" 401 ...	7	62	
" "	647	" 401 ...	7	74	
Guinea-pig	458	" 401 ...	10	61	
" "	672	Rat 477 ...	20	62	
" "	638	Monkey 511 ...	14	80	
Goat ...	512	Situtunga	15(?)	...	Has suffered from abscess on leg, from which it recovered rapidly. Apparently in excellent health after 220 days.
		509-10.			
" ...	579	Monkey 401 ...	?	...	Presence of trypanosomes proved by subinoculation into monkey. Never seen in peripheral blood. Killed after 224 days, showing great weakness; some emaciation.
Dog ...	573	" 401 ...	12	96	Extremely emaciated; some opacity of cornea. Killed when at point of death.
" ...	574	Goat 512 ...	14	43	In very poor condition at the time of inoculation and throughout the experiment.
Calf ...	478	Monkey 401 ...	?	...	Proved by subinoculation into monkey. Trypanosomes seen only once in peripheral blood. Apparently in excellent health after 237 days.

For comparison with the reactions of this Damba trypanosome the following experiments may be considered, involving other trypanosomes strains employed at Mpumu.

Two types were selected for experiment, the one represented by various strains recovered from wild Lake-shore flies by feeding them on monkeys; the other an undoubted *T. gambiense*. This last was obtained in Monkey 199 by direct inoculation from a reed-buck in captivity at the laboratory, 15 months after the original infection of this animal with a human strain. It should be noted that in the case of all the strains employed the trypanosomes

had more or less recently passed through *Glossina palpalis*. This is also presumably the case with the Damba strain as found originally in the wild antelope. This fact is of considerable importance in that it eliminates all idiosyncrasies acquired during continued maintenance in laboratory animals.

Experimental animal.	Origin of trypanosome strain.	Duration of disease.
		days.
Monkey 113 ...	Kibanga flies direct	85
" 56 ...	" " " " " " " "	104
" 563 ...	" " " " " " " "	147
" 597 ...	Namusenyu flies direct	123
" 538 ...	Human strain from Monkey 199 through laboratory-bred <i>G. palpalis</i>	176
" 643 ...	" " " " " " " "	111
" 642 ...	" " " " " " " "	89
White rat 659	Monkey 597 by inoculation	62
" 660	" 563 " " " " " "	66
" 576	" 113 " " " " " "	57
Guinea-pig 690	Human strain from laboratory antelope	50
" 1	Monkey 199 through laboratory-bred <i>G. palpalis</i>	68
" 2	" 113 " " " " " "	81
" 4	" 113 " " " " " "	76

The average duration of the disease in rats (three experiments) is 61 days: in four guinea-pigs 68 days: in seven monkeys 119 days.

From a comparison of the above two tables it will be seen that there is a close similarity in the animal reactions of all the trypanosomes employed. In no instance does the disease in rats approach the virulence assumed by *T. brucei*. There is, therefore, no reason to doubt that the trypanosomes recovered from wild Lake-shore flies and from wild situtunga are indeed *T. gambiense*.

A visit was paid to Sese Islands where Carpenter had recently obtained an infection in a monkey with some 6,000 odd flies. *G. palpalis* were found to be very numerous and many situtunga tracks were seen. The natural conditions on the islands visited are, however, apparently very much less favourable to the perpetuation of sleeping sickness by these animals than is the case with Damba. There are tracts of open country, between and behind the forests which fringe most of the Lake-shore, where situtunga come out to feed and where there are no tsetse. The antelope do not, therefore, enter a fly area immediately upon leaving the shelter of the forest and papyrus as is nearly always the case on Damba.

Six situtunga were shot during the trip. Their blood was examined in the fresh state, by stained films, and by inoculation into goats and monkeys. By the latter method only was a positive result obtained, *T. vivax* appearing in the goat 13 days after the first inoculation of blood. The two monkeys used in these experiments remained negative to daily examinations for 30 days.

With regard to the appearance of trypanosomes in the goat it must be stated that the animal was sent to Sese direct from Entebbe. I was, therefore, only able to make a few preliminary blood examinations before using it for experiment. In the face of former results with *T. vivax* and the fact that the goat was to all appearance quite healthy, there is every reason to conclude that the trypanosomes came from situtunga.

5.—SOME OBSERVATIONS ON *TRYPANOSOMA PECORUM* (BRUCE) AND *T. UNIFORME* (BRUCE).*

By H. LYNTHURST DUKE.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S.
Received August 16, 1912.)

The fact that antelope play a considerable part in nature as a reservoir for *T. gambiense* has now been thoroughly established. Both laboratory and field results agree in confirming the suspicion which for some time past has attached to these animals. In the case of *T. gambiense*, however, there is nothing very remarkable in the observation that the presence of the trypanosome in their blood apparently exerts no harmful effect on the antelope. *T. gambiense* cannot be considered as a fatal trypanosome for ruminants generally. In the case of *T. pecorum*, however, matters are different. This trypanosome, according to Bruce and his collaborators, causes a rapidly fatal disease in cattle and domestic animals generally. Though apparently widely distributed throughout Uganda, nothing definite is at present known concerning its true carrier in nature. The presence of the disease in districts where tsetse are unknown shows that some other agent is capable of conveying this trypanosome. The behaviour of laboratory-bred *Glossina palpalis* as carriers is so uncertain that this fly would appear at most to be a facultative host, though doubtless under favourable circumstances it may play an important part in nature. Again, nothing is known concerning the existence of a natural reservoir for this trypanosome in Uganda, though Kleine has recently obtained species closely allied to, if not identical with, *T. nanum* and *T. congolense* from antelope in the neighbourhood of Tanganyika. The following experiments were undertaken with a view to discovering whether *T. pecorum* is pathogenic to antelope, and at the same time to test the power of these animals to act as a reservoir for the trypanosome:—

A young bushbuck, born at the laboratory in January, 1911, was inoculated with blood of a monkey suffering from *T. pecorum*. After an incubation period of 15 days trypanosomes appeared in the blood of the bushbuck on May 31, 1911, being present for

* Reprinted from the Proceedings of the Royal Society, Ser. B, vol. 85, p. 554.

some days and then disappearing. Clean laboratory-bred *G. palpalis* were then fed upon the bushbuck and afterwards on clean monkeys. These experiments are given in Table I. As will be seen, in only one instance was a positive transmission obtained, and this was unfortunately not entirely satisfactory, as will be explained below.

Table I.

Experiment No.	Period for which flies fed on Bushbuck 55.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.			
	1911.							
112	June 6-13	42	32	39	1	62	—	
129	" 9-13	37	34	37	1	63	—	
135	" 12-14	34	33	34	0	80	—	
247	Aug. 5-12	93	85	90	8	141	—	
253	" 7-12	105	86	91	1	66	+	Fed on Monkey 359, which developed <i>T. pecorum</i> . Cf. <i>infra</i> .
541	Dec. 1-8 ...	109	56	109	0	61	—	
542	Dec. 4-8 ...	94	39	94	0	58	—	

The following cycle experiments were carried out in which animals other than Bushbuck 55 supplied the infecting feeds:—

Table II.

Experiment No.	Period for which flies fed on infected animal.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.
		1st day.	30th day.	Dissected during experiment.	Containing flagellates.		
	1911.						
96	May 31-June 5, on Monkey 152	46	44	46	1	49	—
212	July 19-21, on Calf 33 ...	83	80	82	2	104	—
368	September 6-9, on Monkey 321...	136	124	133	1	108	—
716	April 18-27, on Calf 688 ...	46	42	45	2	82	—
717	April 18-27, on Calf 688 ...	59	53	59	2	82	—

It is unfortunate that in the case of the only (apparently) successful transmission Experiment (253) a possible alternative source for the trypanosome in the monkey was introduced. Owing to a temporary shortage of monkeys monkey Experiment 359, whose blood had been negative for 10 days after the last feeding of Experiment 253 upon it, was taken to the Lake. Box 253 had been dissected, and as no fly was found with flagellates in the proboscis, it was thought that the experiment would necessarily

be negative. It happened that three Bushbuck, A, B and C, were shot, and the citrated blood of two of them, A and B, was inoculated into the monkey, 3 c.c. from each animal. At the same time, 6 c.c., 5 c.c. and 3 c.c., citrated blood of Bushbuck A, B and C respectively were inoculated into Goat 484. This goat remained healthy and examination of two blood films from each of A, B and C proved negative. Trypanosomes identical morphologically with *T. pecorum* appeared in the blood of Monkey 359 on November 13, 1911, 23 days after the inoculation of the bushbuck blood and 33 days after the last feed of Box 253. From November 1 to 10 inclusive the monkey was not examined. The trypanosomes might thus have been derived either from the bushbuck or from the positive fly of Experiment 253; in each case with a long incubation period.

The negative evidence of Goat 484 and of the blood films, together with the fact that such a trypanosome has never before been recovered from wild flies in the Mpumu district, makes it almost certain that the trypanosomes in Monkey 359 came from Fly 9 of Table III. The curious inability of laboratory-bred flies to infect monkeys with *T. pecorum*, even though showing a well marked proboscis infection, has been mentioned by Fraser and myself, and is also evident in Table III.

It is thus possible that the unusual swarming condition of the sucking stomach to be described in Fly 9 of this table may have a definite developmental significance in *T. pecorum*.

It may be noted that the trypanosomes in the monkey were characterised by a large vacuole posterior to the trophonucleus. This was very striking in the fresh state, even with low magnifications. This condition was lost in a subinoculated white rat.

A further observation which to some extent provides a parallel for the long incubation period, supposing Fly 9 to have been responsible for the infection, is afforded by Experiment 644. In the case of this monkey, inoculated from a goat suffering from *T. pecorum*, trypanosomes first appeared in the monkey's blood 26 days after inoculation. The blood in this case was examined daily. It is to be noted also that *T. pecorum* may often not be seen for days in monkeys' blood, and when present is frequently in very scanty numbers.

The curious backward condition of Fly 12 is interesting. Similar instances occurred in flies infected with *T. nanum*,* but then there was a possibility of a secondary "pick up" of trypanosomes from the calf employed. In the present instance, as the monkey of Experiment 247 never became infected, this explanation is not available.

It is to be regretted that in only three cases were the salivary glands obtained in the above positive flies. In all these the glands were negative. In this respect also *T. pecorum* shows resemblances to *T. nanum*.

* "Transmission of *T. nanum*," 'Roy. Soc. Proc.', 1912, B, vol. 85.

Table III.

Date.	No. of fly.	Age of fly when dissected.	Sex.	Expt. No.	Region of gut.					Injection.
					Hind.	Thoracic gut up to proventriculus.	Proventriculus.	Sucking stomach and duct.	Salivary glands.	Proboscis.
1911.										
July 15	1	9	♂	112	++	—	—	0	0	—
Aug. 16	2	11	♀	247	++	—	—	0	0	—
" 29	3	24	♀	247	++	++	—	0	0	—
Sept. 19	4	45	♂	247	++	++	0	0	0	—
" 4	5	45	♀	212	++	0	+	0	0	—
" 4	6	45	♂	212	++	++	++	0	—	—
July 19	7	49	♀	96	++	++	—	0	0	—
Aug. 11	8	63	♀	129	++	++	++	0	0	—
Oct. 10	9	64	♀	253	++	++	++	++	—	Contents of gut and proventriculus injected into 2 white rats = negative.
" 14	10	70	♂	247	++	++	++	0	0	
Nov. 21	11	76	♀	368	+	++	+	0	0	
" 3	12	90	♀	247	++	—	—	0	0	
" 2	13	104	♀	212	++	++	+	0	—	
Dec. 5	14	122	♂	247	++	++	—	—	0	
" 26	15	141	♀	247	++	++	++	—	0	

In the above table the negative sign implies absence of flagellates ; the sign 0 means that no observation was recorded.

A remarkable feature of the above table is the fact that no proboscis infections were obtained before the 76th day of an experiment. This delay on the part of the flagellates in reaching the proboscis became evident during the dissection of the earlier experiments and led to the carrying on of the later boxes for a considerably longer period. In spite of this, however, although four flies were obtained showing a good infection of the proboscis, none of these proved capable of transmitting the trypanosome to a healthy monkey. The only successful transmission, as has been pointed out above, was apparently caused by Fly 9, which showed a swarming infection of the sucking stomach and its duct. This inability on the part of the proboscis-infected flies to infect a monkey is remarkable. On several occasions the flies were starved for short periods and then again fed upon the monkey; also with negative results.

In the case of Fly 9, supposing it to be the infecting agent, two possibilities must be considered. Either the flagellates of the sucking stomach were responsible for the infection, or the proboscis may have shown a temporary infection which at the time of dissection had disappeared.

From the above experiments it will be seen that Bushbuck 55 was still capable of infecting laboratory-bred *G. palpalis* some three months after its original infection with *T. pecorum*.

The following experiments were carried out 10 months after the original infection of the bushbuck:—

Date.	Expt. No.	Quantity of blood.		Result.	Animal used for injection.
		Pure.	Citrated.		
21.3.12 ...	670	c.c. 2½		—	Monkey.
23.3.12 ...	678	6		—	
3.4.12 ...	688	3½		+	Calf.

In considering the positive result of Experiment 688 the following facts may be quoted as excluding any source of error:—

(1) Since my arrival in Mpumu in September, 1910, there has been no case of spontaneous infection with this trypanosome among the laboratory cattle.

(2) The cattle in the neighbourhood of the hill are apparently free from the disease.

(3) A calf of about the same age as Experiment 688 (both were born at the laboratory) has been under daily blood examination from the beginning of April until the date of writing, and has never developed trypanosomes. These two animals have never been allowed to leave the hill top, and have always been stalled together.

It will thus be seen that no local epidemic of *T. pecorum* disease, such as affected the transmission work of the 1908—1910 Commission, interfered with the above experiments.

The interesting result obtained in Experiment 688 proves that *T. pecorum* can exist in antelope for at least 323 days. During this time it apparently exerts no harmful effect upon the host. The bushbuck, Experiment 55, is to all appearances in excellent health, and has been growing rapidly throughout the period covered by these experiments.

An interesting point also is the rapid course of the disease in Calf 688, which died in 51 days. Previous to the introduction of the trypanosome into the bushbuck there were some signs that the organism was losing its influence, possibly owing to continued maintenance in laboratory animals. Thus Calf 33, which was inoculated with the old laboratory strain of *T. pecorum* from a monkey, first showed trypanosomes on May 13, 1910. The animal is still alive and in good condition, and was showing *T. pecorum* in its blood on August 29, 1910; since then the blood has not yet been examined. Bushbuck 55 was inoculated from the same monkey as Calf 33.

There is, of course, the possibility that the extraordinary course of the disease in Calf 33 is due to some immunity peculiar to this individual. Previously, all calves inoculated with this laboratory strain had died of an acute disease.

It will be noticed, however, that the long sojourn in the bushbuck did not render the trypanosome more suited to development in *G. palpalis*, in contrast to the behaviour of *T. gambiense* under similar circumstances.

Trypanosoma uniforme.

As has been pointed out elsewhere, this organism appears to be the most common antelope trypanosome in the Mpumu neighbourhood. According to Bruce and his collaborators, it causes a fatal disease in domestic ruminants, the average duration of the disease in three laboratory-infected goats being 29 days.

My experience of this trypanosome, extending over some 20 months, has not confirmed this opinion. *T. uniforme* has not proved in any way a fatal trypanosome during that time. In only one case has animal died of an unequivocal *T. uniforme* infection, this being apparently the case with Calf 481, although for months before death no trypanosomes were visible in the peripheral blood.

A characteristic feature of *T. uniforme*, which is especially marked in goats, is the manner in which, after a few weeks, it totally disappears from the peripheral blood as regards ordinary routine examination, while the animal shows no symptoms whatever.

When, however, this trypanosome occurs together with *T. vivax*, or possibly with *T. gambiense*, the animal may become rapidly emaciated, and die with parietic symptoms, particularly in the hind limbs. This, however, is not a constant phenomenon, as is shown in the case of Goat 512, which was infected in

November, 1911, simultaneously with *T. uniforme*, *vivax*, and *gambiense*, and at the time of writing is apparently in perfect health.

The following table is of interest, as affording marked contrast to the course of disease described by Bruce:—

Experiment.	Infected.	Duration of disease.	Remarks.
Goat 352 ...	1910. Nov. ...	Killed Dec., 1911 ..	In apparently perfect health at time of death.
" 397 ...	1911. Jan. ...	" " ...	" " "
" 431 ...	Feb. ...	" " ...	" " "
Calf 481 ...	Jan. ...	Died in July, 1912, after 18 months.	No trypanosomes visible during last week. Slight emaciation.
" 536 ...	Feb. ...	" " " " "	Killed by leopard. Apparently in excellent health when attacked.
" 573 ...	Mar. ...	" " " " "	Alive and well, July, 1912.
" 616 ...	" ...	" " " " "	" " " " "
Bushbuck 620	Jan. ...	" " " " "	In excellent health, July, 1912.
Sheep 534 ...	" ...	Up till date of infection with <i>T. gambiense</i> showed good health.	Infected with <i>T. gambiense</i> in Feb., 1912. Died May, 1912.

In all the above experiments, the strain employed was derived originally from a wild bushbuck shot at the Lake-shore.

The strain employed by Bruce and his collaborators was derived originally from oxen, which may explain the difference in the virulence.

Antelope as a Reservoir of T. uniforme.

Two situtunga which were brought alive to the laboratory were found to harbour this trypanosome as a natural infection.

These animals have been kept under observation, and from time to time laboratory-bred *G. palpalis* have been fed upon them. These experiments are summarised in the following table:—

Expt. No.	Period flies fed on Antelope.	Number of flies.				Duration of experiment in days.
		1st day.	30th day.	Dissected.	Containing flagellates.	
464	Oct. 10-18, 1911, on Situtunga 173	57	47	55	0	41
528	Nov. 21-28 " " 173	104	84	104	2	55
529	" 22-28 " " 173	93	62	93	0	55
706	Apr. 11-17, 1912 " 173	86	58	86	3	50
707	" 12-17 " " 173	61	47	61	2	49

In all the positive flies of the above experiments trypanosomes were only found in the proboscis.

It is impossible to know when Situtunga 173 became infected, but reckoning from its arrival at the laboratory on June 25, 1911,

it will be seen that the animal was still capable of infecting laboratory-bred *G. palpalis* with *T. uniforme* after a period of 10 months.

During this time the animal has remained in excellent health, though confined under by no means ideal conditions.

The question of wild antelope in tsetse regions serving as a trypanosome reservoir thus becomes increasingly important. In this Protectorate alone *T. gambiense*, *vivax*, *pecorum*, and *uniforme* have all been proved capable of surviving for a considerable time in these animals, apparently without exerting any injurious effect upon their hosts.

In other parts of Africa *T. rhodesiense*, *brucei*, *congolense*, and *nanum* have been recovered from game.

This is not the place for a discussion on the significance of the problem from an administrative point of view. Extermination of the game in Africa is a colossal undertaking, and until something more is known concerning the rôle played by birds and reptiles in the spread of trypanosomiasis it would seem inadvisable to set too much faith in this drastic measure.

6.—A CAMEL TRYPANOSOME, WITH SOME REMARKS ON THE BIOMETRIC METHOD OF DIAGNOSING TRYPANOSOMES.*

By Dr. H. L. DUKE.

(Communicated by Sir John Rose Bradford, K.C.M.G., Sec. R.S.
Received September 2, 1912.

The trypanosome which forms the subject of the following experiments was kindly forwarded to Mpumu by Mr. E. Montgomery, M.R.C.V.S., from the veterinary pathological laboratory, Nairobi. The organism was originally obtained from the blood of a camel from Boran. Experiments were undertaken to see whether the trypanosome was transmissible by laboratory-bred *G. palpalis*, and a few sub-inoculations were performed.

Morphology.—Length: 400 trypanosomes taken at random were measured, and the results are given in Table I. As is there shown, the length varies between 18 μ and 34 μ .

Shape: The great majority of the trypanosomes seen are slender; a few forms occur which are markedly broader. The flagellar end may be very much drawn out, the kinetic nucleus being sometimes from 4 μ to 4.5 μ from this extremity.

Undulating membrane: Well developed.

* Reprinted from the Proceedings of the Royal Society, Ser. B, vol. 85, p. 563.

Flagellum: In the slides examined, only one single specimen was observed in which there could be any doubt as to the presence of a free flagellum. In the majority, the free portion of the flagellum is very well marked.

Kinetic nucleus: Always clearly discernible; small and round, situated either almost in contact with the posterior end, or at varying distances from this extremity up to $4\cdot5\ \mu$.

Nucleus: Situated near the middle of the body.

The following Table I. gives the measurements from four experimental animals. 100 examples were drawn from each experiment:—

Table I.*

Expt.	18 μ .	19 μ .	20 μ .	21 μ .	22 μ .	23 μ .	24 μ .	25 μ .	26 μ .	27 μ .	28 μ .	29 μ .	30 μ .	31 μ .	32 μ .	33 μ .	34 μ .	Average.
Rat 780 ..			6	7	11	11	7	8	7	10	8	8	10	6	3			25.1
Rat 696 ..			1	3	4	7	9	5	5	15	15	9	13	8	5		1	27.0
Monkey 646 ..			5	4	8	8	6	11	18	13	16	2	7	2				25.5
Dog 671 ..	1	1	6	6	12	11	11	17	12	15	2	2	4					24.4
Totals ..	1	1	18	20	35	37	33	39	42	53	41	21	34	14	8	2	1	
Per- centages.	0.2	0.2	4.5	5	8.7	9.2	8.2	9.7	10.5	13.2	10.2	5.2	8.5	3.5	2	0.5	0.2	

* In the table of measurements of the antelope trypanosomes as printed in my paper earlier in this volume (p. 166), two members are omitted, viz., in column 18μ on line (2) 511, monkey, the figure 1 is omitted, and in the line of totals the figure 7 is omitted.

The fly experiments may now be briefly summarised. On no occasion was a successful transmission obtained and no flagellates were found in the flies employed, either in proboscis or gut:—

Table II.

Experiment No.	Period on infected animal.	Number of flies.				Duration of experiment days.	Result.
		1st day.	30th day.	Dissected.	Containing flagellates.		
680	Mar. 27-30 on Monkey 676	48	35	48	0	33	—
681	" 28-30 " 676	42	29	42	0	32	—
683	" 31-Apr. 4 on Dog 671	82	56	82	0	30	—
684	Apr. 1-6 " 671	47	26	47	0	29	—
687	" 2-6 " 671	62	43	62	0	52	—
693	" 8-15 " 671	79	47	79	0	45	—
698	" 10-15 on Monkey 676	73	40	73	0	41	—
	Totals	433	276	433	0		

Animals Susceptible to this Trypanosome.—In the following table those experiments marked* were performed by Mr. Montgomery, and I wish to express my indebtedness to him for the

permission to quote them. It will be noticed that the disease is rapidly fatal to rats and monkeys, while in dogs it is relatively slow:—

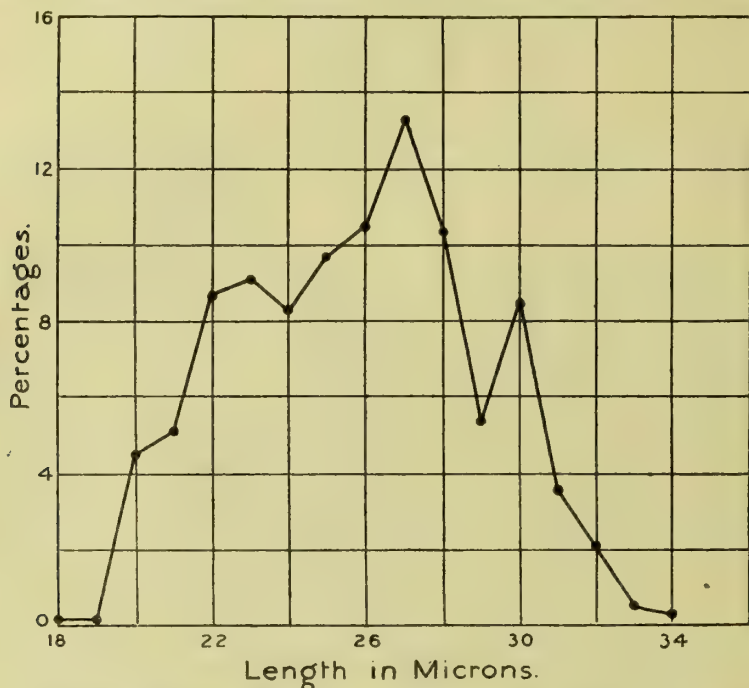
Table III.

Expt. No.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
		Days.	Days.	
*Ox 123 ...	Camel 8 ...	?	112	Trypanosomes never seen in peripheral blood; proved by subinoculation.
*Mule 6 ...	Dog 3 ...	5	...	Alive after 130 days; not showing by direct observation.
* " 25 ...	Donkey 22...	?	136	
*Donkey 13 ...	Dog 3 ...	8	99	
* " 22 ...	Donkey 13...	8	128	
*Dog 3 ...	Camel 8 ...	11	63	
* " 5 ...	Ox 123 ...	10	40	
* " 6 ...	Dog 3 ...	8	21	
* " 14 ...	Mule 6 ...	7	34	
* " 21 ...	} " ...	?	...	{ Alive after 150 days; some emaciation; no corneal changes.
* " 671 ...				
* " 29 ...	" ...	10	62	
* " 30 ...	Donkey 22...	7	83	
Monkey 676...	Dog 671 ...	4	26	Old monkey at commencement of the experiment.
White rat 696	Monkey 676	4	10	
" 697	" "	4	10	
" 674	Dog 671 ...	3	10	
" 675	" ...	3	5	
" 780	Goat 677 ...	?	14	
Goat 677 ...	Dog 671 ...	?	...	Trypanosomes never seen in peripheral blood. Alive and in good condition after 110 days.
Guinea-pig ...	" ...	4	85	
" ...	" ...	4	70	

Identity of the Trypanosome.—The diagnosis appears to rest between the trypanosomes of the surra-nagana group, *e.g.*, *T. brucei*, *evansi*, *equiperdum*, and *equinum*. The well marked kinetic nucleus seen in stained films excludes *T. equinum*, and the absence of any of the characteristic plaque lesions of dourine is against *T. equiperdum*.

There remain *T. brucei* and *T. evansi* to be considered. The following curve constructed from Table I. corresponds roughly to Bruce's curve for *T. evansi*, and the absence of any short stumpy forms from all the experimental animals examined is against *T. brucei*. Thus out of 400 specimens measured in the present instance no trypanosomes were seen measuring less than 18μ in length, while Bruce describes a considerable number of examples of *T. brucei* between 13μ and 17μ . Turning to the table of animal reactions little or no assistance is forthcoming in deciding between *T. brucei* and *T. evansi*. In both species the disease

is typically a very rapid one in white rats, and the number of trypanosomes in the peripheral blood shortly before death is enormous. The course of the disease in dogs in the above table is not characteristic of either *T. brucei* or *T. evansi*, which are rapidly fatal to these animals. In the case of surra, however,



the disease appears less rapid in its course than with nagana. Thus Laveran quotes experiments of Lingard's in which dogs lived 27½, 29, 34, 36, 47, 97 days after inoculation with *T. evansi*, the last having been inoculated from a naturally infected bovine. In the case of *T. brucei*, however, the same authority gives 26 days as the maximum duration of nagana in dogs.

Though by no means a typical *T. evansi* it is plain that the above experiments suggest this trypanosome rather than *T. brucei*, and this conclusion is supported by the evidence of morphology.

I shall take this opportunity to consider briefly the question of the biometric method of diagnosing trypanosomes from the point of view of a worker dealing with the problem in the field. Of late a great deal of attention has been devoted to this method, with the result that there appears to be some danger of its attaining undue importance. In certain cases it is of undoubted value, as in discriminating between two such species as *T. vivax* and *T. uniforme*, where the difference in size is one of the most important points of distinction. Here, however, it will not be necessary to measure a great number of trypanosomes. In the case of other mammalian trypanosomes, *e.g.*, *T. brucei*, *gambiense*, *pecaudi*, *rhodesiense*, *evansi*, *equiperdum*, *equinum*, *nanum*, and *congolense* exhaustive measurements are of little use in diagnosis.

The following example will illustrate very well both the uses and the limitations of the measurement method. In papers dealing respectively with the morphology of *T. gambiense* and *T. evansi* Bruce gives three curves of *T. brucei* for purposes of comparison. The apices of the curves are at $18\ \mu$, $20\ \mu$, and $24\ \mu$ respectively, a very considerable variation. Further, in dealing with *T. evansi*, he claims that from a comparison of the two curves it is possible to distinguish the two species from one another. In the case of *T. brucei* the bulk of the curve will be between $13\ \mu$ and $35\ \mu$, with *T. evansi* between $18\ \mu$ and $30\ \mu$. A glance at the curves, however, will reveal the fact that the real difference between the two trypanosomes as determined by the biometric method is that *T. evansi* shows no forms of less than $18\ \mu$ in length. In other words, if in the course of examining a slide a trypanosome is found below say $15\ \mu$ in length, the diagnosis cannot be *T. evansi*. Thus the diagnosis has in reality turned upon the minimum measurements of the two species.

The measurements quoted for the different species of trypanosomes vary with the observer, even although the staining and fixing methods are the same in every case. This variation is due to a number of factors, the least important of which is probably the technique employed in the actual measurement.

It would appear from Miss Robertson's recent work on *T. gambiense** that an endogenous cycle exists in the mammal, the duration of which is variable and incalculable, the result being that the type of trypanosome predominating in the peripheral blood is constantly changing. The date of the infection has no constant connection with the course of the phenomenon. In each cycle the change would appear to be from short to long forms, returning to the short forms again before the temporary disappearance from the blood.

To obtain an accurate conception of the dimensions of any trypanosome it is plain that all the stages of such a cycle must be followed out, from its commencement to its conclusion. Slides taken haphazard from time to time are useless for comparative purposes, as practically any type of curve may be obtained according to the stage of the cycle. The three curves of *T. brucei* quoted above from Bruce's paper are an instance of this. In a recent report summarised in *Sleeping Sickness Bulletin* 36 (vol. 4, p. 145), Dr. Stephens lays great stress on the minute technique of measurement, and points out that Bruce's method is open to criticism. The contention advanced here is that such a criticism, though doubtless true as regards geometrical exactitude, is irrelevant as regards its practical application. The errors involved are so small that they can have no serious effect in the matter of diagnosis, when all the other aspects of the question are considered. Dr. Stephens does not apparently criticise the method of preparing the slide adopted by Bruce, he merely asserts that the actual estimate may err within fractions of a micron. Surely such a source of error cannot be very important

* 'Roy. Soc. Proc.,' B, vol. 85 (No. 582).

in plotting out a curve whose unit is 1μ , considering, for example, that all values between 5.5μ and 6.5μ will necessarily be registered as 6μ .

Again, there are other considerations which tend to make the biometric method of diagnosis at best inconstant:—

(1) Fixation will vary in different parts of the same slide and in the preparation of slides by different observers.

(2) Numerous varieties of strain exist among trypanosomes of any species.

(3) The great similarity between many so-called species as regards their length variation.

(4) Probably continued maintenance in laboratory animals leads to slight alterations in the morphology of a strain, which to be kept true should from time to time be passed through its insect host. Thus the age of a strain after its last passage through the appropriate intermediate host may be a very important factor in determining its morphology.

In the face of such objections it would appear that, although the biometric method may have a certain value in diagnosis, such refinements as suggested by Dr. Stephens are by no means essential. What is required is that measurements be made in as constant a manner as possible. Dr. Stephens' method, involving as it does a complicated projection apparatus, is quite impossible for workers in the field. This in itself is an objection which would seriously neutralise undoubted advantages. It is, however, difficult to believe that the ultimate results of the application of both methods to the same slide would differ to any appreciable extent. Granting, however, for the sake of argument Dr. Stephens' contention, it must still be remembered that its occasional employment will introduce yet another variable factor into this method of diagnosing trypanosomes.

7.—SOME EXPERIMENTS WITH ARSENPHENYLGLYCIN AND *TRYPANOSOMA GAMBIENSE* IN *GLOSSINA* *PALPALIS*.*

By H. LYNDBURST DUKE.

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The experiments detailed below were devised with a view to investigating the action of arsenic in the form of arsenphenylglycin upon *Trypanosoma gambiense* as carried by *Glossina palpalis*. It was found convenient to deal with the subject under four separate enquiries—

I. Does the presence of arsenic in the blood ingested by a positive fly destroy the trypanosomes in that fly?

* Reprinted from Proceedings of the Royal Society, Ser. B, vol. 86, p. 19.

II. Does preliminary feeding of flies on blood containing arsenic have any effect on the subsequent development of trypanosomes in their interior?

III. If flies are fed on blood containing arsenic shortly after the infecting feeds on a *gambiense* monkey, are the flagellates still capable of development in the fly? If they can still develop, is the resultant strain arsenic-resistant in the blood?

IV. Has arsenphenylglycin any prophylactic action against the bite of a fly infected with *T. gambiense*, and, if so, what is the extent of this protection?

In all these experiments it was deemed advisable to feed each box of flies for two consecutive days on the monkey in order to make certain, if possible, that each fly fed. For the same reason special attention was given to each box of flies handled, with the result that it was found that flies reluctant to feed in the morning would often, if given another opportunity some hours later, bite with greater readiness. In spite, however, of all precautions, it is impossible to be absolutely certain on this point. In the great majority of cases the flies fed readily every day.

I. *Does the Presence of Arsenic in the Blood imbibed clean an Infected Fly of its Flagellates?*

To answer this query boxes of laboratory-bred *G. palpalis*, known to be infective with *T. gambiense*, were placed upon monkeys which had previously received a subcutaneous dose of arsenphenylglycin, 0.1 grm. per kilogramme body weight. The experiments were commenced 24 to 48 hours after the administration of the drug, and subsequently the period of time was increased. No change in the flagellates was discernible if the interval between the giving of the arsenic and the feeding exceeded 72 hours. The flies were dissected by Miss Robertson at varying periods after the feeds upon the arsenic monkey. I have here to express my indebtedness to her for the description of the flagellates found. It was constantly noticed in the case of flies which had fed upon a recently inoculated monkey that the walls of the intestine were unduly brittle and friable, and clean dissection very difficult. This was to a great extent avoided by starving such flies for several days after the ingestion of the arsenic blood before dissecting them. In the following experiments various changes were noted in the flagellates in the fly, due presumably to the action of the drug in the blood imbibed:—

Experiment 29.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Oct. 1-2 ...	Fed on Monkey 418 24-48 hours after administration of 0.1 grm. arsenphenylglycin per kilogramme.	
„ 3 ...	Starved	1 fly dies ; nil.
„ 4 ...	Fed on clean Monkey 502	Monkey 502 becomes infected. 2 flies die ; nil.
„ 5 ...	„ „ 427	Monkey 427 becomes infected. 1 fly dies ; nil.
„ 6 ...	Starved and dissected	1 + fly found.

Description of the positive fly:—

Gut: Shows flagellates very much modified, with swollen posterior end; many dead.

Proventriculus and thoracic gut: No flagellates seen.

Salivary glands: Normal, though somewhat slight infection.

It will be noted that for two consecutive days after feeding on the arsenic monkey this fly was infective to monkeys. The condition in this fly affords strong confirmation of the theory that the salivary gland flagellates are the true infecting form.

Experiment 74.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Oct. 24-25 ...	Fed upon Monkey 488 24-48 hours after the administration of arsenophenylglycin, 0.1 grm. per kilogramme.	
„ 26 ...	Starved	9 flies die, among which is + Fly No. 1.
„ 27 ...	Fed on cock.	
„ 28 ...	„ „	2 flies die, one negative, the other + Fly No. 2.
„ 29-Nov.1	„ „	
Nov. 2-4 ...	„ clean Monkey 503... ..	Monkey 503 became infected. 1 fly dies; nil.
„ 5-6 ...	Starved and dissected	+ Fly No. 3 found.

Description of the three positive flies:—

Fly 1. *Gut*: Few altered flagellates, all dead, with swollen posterior ends, and generally altered.

Proventriculus: Nil.

Salivary glands: Only one gland obtained; this contained living and apparently quite normal flagellates.

Fly 2. *Gut and Proventriculus*: Show no flagellates, living or dead.

Salivary glands: Contained flagellates ++ +, actively motile.

Fly 3. *Gut*: ++ +, apparently normal.

Proventriculus: Nil.

Salivary glands: ++ +, normal.

Whether the condition of this fly is due to the original gut flagellates having escaped destruction by the arsenic, or whether in the 10 days which elapsed between the arsenic feeds and dissection the gut became reinfected from the salivary glands, cannot be decided. In relation to the first alternative it is improbable that a fly would refuse to feed upon two successive days unless at the point of death.

The mortality after the arsenic feeds should be noted in this and other similar experiments.

Experiment 357.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Oct. 10-11 ...	Fed on Monkey 452 24-48 hours after administration of arsenphenylglycin, 0.1 grm. per kilogramme.	
„ 12 ...	Starved	7 flies die; nil.
„ 13 ...	Dissected	1 + fly found.

(This box was killed by wood-smoke instead of the usual chloroform.)

Description of the positive fly:—

Gut: Flagellates all dead, shape considerably altered, the posterior end being frequently swollen up to a marked degree.

Salivary gland: Numerous flagellates, but all dead; shape perfectly normal.

Apparently this curious condition of the gland flagellates was due to the smoke. The effect of this treatment is very marked; the glands show a dark granular appearance very different from the clear transparent state to be observed in normal specimens. This darkening of the glands proved by subsequent experiments to be a characteristic of smoke-killed flies. This condition has never been observed in flies killed with chloroform.

Note the mortality after the arsenic feeds.

Experiment 383.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Oct. 24-25 ...	Fed on Monkey 487 24-48 hours after the administration of 0.1 grm. arsenic per kilogramme.	
„ 26 ...	Starved.	
„ 27-28 ...	Fed on Monkey 493 24-48 hours after arsenic 0.1 grm. per kilo.	
„ 29 ...	Starved.	
„ 30 ...	Fed on cock	26 flies die; nil.
„ 31-Nov. 2	Fed on clean Monkey 502 (a) ...	Monkey became infected 1 fly dies; nil.
Nov. 3 ...	Starved	1 fly dies; nil.
„ 4 ...	Starved and dissected	+ Flies Nos. 1 and 2 found

Description of the two positive flies:—

Fly 1. *Gut and proventriculus*: Devoid of flagellates, alive or dead.

Salivary gland: + + +, with normal active flagellates.

Fly 2. *Gut*: + +, no alteration in form observable.

Foregut: +, no alteration in form observable.

Proventriculus: Nil.

Salivary gland: + + +, normal.

In this experiment the flies were afforded an opportunity of a second feed of arsenic blood. In spite of this the salivary flagellates—the infecting form—were, it appears, unaffected. Apparently fly No. 2 illustrates re-infection of the gut from the salivary gland, as it is very improbable that any of the original gut flagellates would survive the double feeding on arsenic blood.

Note the mortality after the arsenic feeds.

Experiment 358.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Oct. 10-11 ...	Fed on Monkey 438 24-48 hours after administration of arsenophenylglycin, 0.1 grm. per kilogramme.	
„ 12 ...	Starved.	
„ 13 ...	Dissected	+ Flies Nos. 1, 2, 3, 4 found.

Description of the four positive flies:—

Fly 1. *Gut* (containing no blood): + + + active, normal.
Proventriculus: +, normal.
Salivary glands: + + +, active and normal.

This fly showed no sign of blood in its gut, and therefore presumably did not feed on Monkey 438.

Fly 2. *Gut* (empty forward with a little pale fluid posteriorly): Some tendency towards posterior swelling, but still active.
Salivary glands: + + +, active and normal.

Fly 3. *Gut* (containing altered blood): + + +, active, but some show posterior swelling.
Salivary gland: + + +, apparently normal.

Fly 4. *Gut* (containing altered blood): + + +, few dead, many altered in shape but still slowly motile, others active.

The flagellates of these flies show less effect from the arsenic than is usual at this period.

It may be remarked, as justifying the conclusion that the above departures from the normal in the flagellates are due to the action of the drug, that such alteration in form and motility have never before been seen in positive flies.

The dead flies are removed every morning, and in the normal course of events the flagellates are always seen to be actively motile with no such morphological changes as those described above. Moreover, in cases in which the normal flies have been left until the flagellates are moribund, death occurs without the characteristic swelling of the posterior part always to be observed in the presence of arsenic.

Experiment 550.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Jan. 31–Feb 1	Fed on Monkey 632 48–72 hours after administration of arsenphenylglycin, 0.1 gm. per kilogramme.	
Feb. 2 ...	Starved.	
„ 3 ...	Dissected	+ Flies 1 and 2 found.

Description of the two positive flies:—

Fly 1. Gut + + +, normal (no traces of blood seen).

Salivary glands: + + +, normal.

Fly 2. Gut: + +, a few are altered as described above.

Salivary gland: + + +, normal.

A very slight effect appears to have been exerted in Fly No. 2 by the arsenic. Fly No. 1 either fed very slightly or not at all on Monkey 632.

Experiment 567.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Jan. 20–21 ...	Fed on Monkey 620 48–72 hours after administration of arsenphenylglycin, 0.1 gm. per kilogramme.	
„ 22–26 ...	Starved.	
„ 27 ...	Dissected	1 + fly found.

Description of the positive fly:—

Hindgut: Nil.

Posterior part of thoracic gut: Normal, + + +.

Anterior part of thoracic gut: Nil.

Proventriculus: Nil.

Salivary gland: + + +, normal.

From the above experiments it will be seen that the flagellates in the gut of flies fed upon a monkey within 24 to 48 hours of the administration of arsenphenylglycin in doses of 0.1 gm. per kilogramme are markedly affected. The flagellates in the salivary glands are apparently not injured in any way, nor does the fly lose its power of infecting. This evidence supports the theory that the salivary gland flagellates are the normal infecting agents. In a paper shortly to be published, Miss Robertson brings forward a further mass of evidence to support this conclusion. There is no reason to doubt that the secretion of the salivary gland is poured out into the wound made by the fly's proboscis at the commencement of the act of feeding. Whether or not this process is repeated during the course of feeding cannot well be determined. These functions of the salivary gland and its contained flagellates are well borne out by the interrupted feeding experiment to be referred to shortly.

It is plain that any attempt to clean a positive fly of its flagellates by feeding it upon an animal whose blood contains arsenic will fail, as the gland flagellates will not come into contact with the drug.

II. *Has the Preliminary Feeding of Flies on Arsenic-containing Blood any Effect on the Subsequent Development of the Flagellates in their Interior?*

The first pair of experiments, Nos. 336 and 337, devised to elucidate this point proved fruitless, as no positive flies were found either in the arsenic box or its control.

Experiment 712.

Date.	Day of expt.	Procedure.	Remarks.
Apr. 15 ...	...	Fed on Monkey 708 48 hours after the administration of arsenic, 0.1 grm. per kilogramme.	
" 16-17	1-2	Fed on Monkey 711 24-48 hours after the administration of arsenphenylglycin 0.1 grm. per kilogramme.	
" 18 ...	3	Starved.	
" 19-20	4-5	Fed on Monkey 597, which shows <i>T. gambiense</i> +.	
" 21 ...	6	Starved.	
" 22-	7-35	Fed on cock.	
May 20.			
May 21-22	36-37	Starved and dissected ...	3 + flies found out of 112 dissected = 2.6 per cent.

Experiment 713 (control).

Date.	Day of expt.	Procedure.	Remarks.
Apr. 15-17	1-2	Fed on normal monkey.	
" 18 ...	3	Starved.	
" 19-20	4-5	Fed on Monkey 597 (<i>T. gambiense</i> +).	
" 21 ...	6	Starved.	
" 22-	7-35	Fed on cock.	
May 20.			
May 21-22	36-37	Starved and dissected ...	8 + flies found out of 69 dissected = 11.5 per cent.

From these two experiments, as far as any conclusion can be drawn from such limited evidence, it would appear that the initial arsenic feeds influence unfavourably the subsequent development of the flagellates in the fly.

III. Does Feeding on Arsenic-containing Blood, immediately after the Infecting Feeds, prevent the Subsequent Development of Flagellates in the Fly, or does it result in the Production of an Arsenic-fast Strain?

Experiment 416.

Date	Day of expt.	Procedure.	Result.
Sept. 30- Oct. 1.	1	Fed on Monkey 199 (<i>T. gambiense</i> +).	Monkey 426 received 0.1 grm. per kilogramme arsenphenylglycin on Oct. 3. It eventually became infected.
Oct. 2 ...	2	Fed on cock.	
" 3 ...	3	Starved.	
" 4- Nov. 20.	4-51	Fed on Monkey 426	
Nov. 21-22	52-53	Starved and dissected	1 + fly out of 82 dissected = 1.2 per cent.

Monkey 426 became infected on November 27. On December 4, when trypanosomes were showing + + + +, it received 0.1 grm. arsenphenylglycin per kilogramme. Trypanosomes disappeared the following day and were not seen again, although the monkey was examined daily until death on January 9.

Experiment 417.

Date.	Day of expt.	Procedure.	Remarks.
Sept. 30- Oct. 1.	1	Fed on Monkey 199 (<i>T. gambiense</i> +).	Monkey 494 becomes infected.
Oct. 2 ...	2	Fed on cock.	
" 3 ...	3	Starved.	
" 4-25...	4-25	Fed on cock.	
" 26- Nov. 9.	26-40	Fed on Monkey 494	3 + flies out of 103 dissected = 2.8 per cent.
Nov. 10-21	41-52	Fed on cock.	
" 22-24	53-55	Starved and dissected	

It is possible that the + fly in Experiment 416 did not feed upon Monkey 426 on the first day after the arsenic feeding. The strength of the drug to which the newly-imbibed trypanosomes were subjected would thus be reduced. In any case, the trypanosomes in Monkey 426 at the end of the experiment showed no resistance to arsenic.

Experiments 428 and 429, in each of which 89 flies were employed, and Experiments 350 and 351, in which 121 and 77 flies were used respectively, were useless, as no flagellates were found in either the arsenic boxes or their control.

Experiment 714.

Date.	Day of expt.	Procedure.	Remarks.
Apr. 17-18	1	Fed on Monkey 597 (<i>T. gambiense</i> +).	
" 19 ...	2	Starved.	
" 20-	3-33	Fed on cock.	
" May 20.			
May 21 ...	34	Dissected	7 + flies found out of 61 dissected = 11.4 per cent.

Experiment 715 (control).

Date.	Day of expt.	Procedure.	Remarks.
Apr. 17-18	1	Fed on Monkey 597 (<i>T. gambiense</i> +).	
" 19 ...	2	Starved.	
" 20-25	3-8	Fed on cock.	
" 26-27	9-10	Fed on Monkey 724	Monkey 724 received 0.05 grm. per kilogramme arsenphenylglycin on April 25.
" 28 ...	11	Starved.	
" 29 ...	12	Fed on Monkey 724	
" 30-	13-33	Fed on cock.	
" May 20.			
May 21 ...	34	Dissected	1 + fly found out of 58 dissected = 1.8 per cent.

This positive fly showed only a slight gut infection; no flagellates were present in salivary glands or proventriculus. This is an extremely backward state of development for the age of the fly.

From these experiments it appears that ingestion of arsenic blood immediately after the infecting feeds checks subsequent development of flagellates in the fly.

IV. To Investigate the Prophylactic Properties of Arsenphenylglycin against the Bite of *G. palpalis* Infected with *T. gambiense*.

In a paper summarised in the *Sleeping Sickness Bulletin*, No. 24, vol. 13, Mesnil and Kérandel give some experiments dealing with the prophylactic action of arsenphenylglycin against inoculation of *T. gambiense* into monkeys. The following experiments were undertaken on similar lines, but only the smaller doses used by the French observers were employed. Thus in the first series 0.1 grm. of the drug per kilogramme were inoculated subcutaneously; in the second 0.05 grm. per kilogramme. Such proportions when applied to man involve a relatively enormous dose of the arsenphenylglycin. Nevertheless, although the practical application of the results given below may be very limited, the much greater potency of the drug against the fly infection is of considerable interest.

In undertaking such experiments with living flies an obvious difficulty arises in the uncertainty as to whether the infected fly has fed upon the experimental animal. To insure this as far as possible without at the same time vitiating the exactness of the experiment, each box of flies was placed upon the monkey for two

consecutive days, and on each day repeated efforts were made if the flies showed reluctance to feed. In the great majority of cases a day's starvation before the experiment commenced insured vigorous feeding of all the flies. Again, considerable time must often elapse before an infected box of flies is obtained, a large number of boxes having to be rejected after some 30 days' observation, owing to the flies proving non-infective. It was thus found necessary on several occasions to employ a positive box for several different experiments. Also, owing to the great sacrifice of experimental animals involved in testing the infectivity of each box, it was sometimes found necessary to employ untested boxes, relying on the ultimate dissection of the flies to prove their infectivity. Fortunately two monkeys were available which proved highly infective to flies. Thanks to a large number of experiments carried out by Miss Robertson, in which these two animals were employed for the infecting feeds, it became evident that flies infected from either of these monkeys were invariably infective by the 30th day of the experiment, and often several days earlier. This fact, together with the observation derived from Miss Robertson's experiments—that the infectivity of the fly coincided with the invasion of the salivary glands by the flagellates—made it possible to employ untested boxes as above described. All such boxes were kept until the 32nd day, and then assumed to be infective. If on subsequent dissection no + flies were found, then the box was ignored. In the following tables it is stated whether or not a tested box was employed. It will be noted that in positive Experiment 757 only untested boxes were used.

The following facts must also be considered as having an important bearing on the present series of experiments:—

1. The mere introduction of the proboscis of a positive fly into the skin of its victim can produce infection. This was proved by Fraser and myself by an experiment in which interrupted feeding was employed, and is in agreement with the contention that the salivary gland flagellates are responsible for infection. Thus a fly need not have actually extracted any blood to have infected the monkey.

2. A single positive fly can infect a clean monkey on three consecutive days. This experiment was reported by me to the Royal Society in April, 1912. This applies to the occasional employment of the same box in different experiments on consecutive days.

3. The facts recorded in Section I relating to the persistence of the salivary gland flagellates and the infecting power of a fly, even after the gut has been cleared by arsenic feeding.

The actual experiments may now be considered. The headings of the columns are self-explanatory. The variation in the number of positive flies on the two days of an experiment is due to the necessity of apportioning the available positive boxes, so that each monkey had every chance of infection. In many cases boxes had to be ignored owing to their proving negative on dissection. Each monkey was kept under observation for a considerable time in consideration of the enormous incubation period reported in certain experiments by Mesnil and Kérandel where trypanosomes appeared only after 39 days. No such phenomenon occurred here.

Expt. No.	Period elapsing between the administration of the arsenic and the application of the positive flies.	Number of experiments fed on the monkey.		Number of positive flies to which the monkey was exposed.		Duration of the examina- tion of the monkey.	Result.	Remarks.
		First proved infective on a clean monkey.	Not so tested.	First day.	Second day.			
418	Hours.	1		1	1	Days. 52	—	
438	24-48	1		4	4	Died after 9 days.	?	Received second dose of approximately 0.07 grm. per kilogramme three days after first dose. Died of arsenic poisoning.
452	24-48	1		1	1	45	—	
487	24-48	1		2	2	40	—	
493	24-48	1		2	2	41	—	
524	48-72	1		2	2	11	?	
620	48-72	1		1	1	50	—	
632	48-72	1		2	2	60	—	Old monkey ; showed severe local reaction ; killed.
654	72-96	1		1	1	60	—	
694	72-96	2		3	3	62	—	
661	96-120	1		6	6	50	—	
662	96-120	1		2	2	42	—	These two flies died on 22nd, and, from the condition of their gut, apparently had not fed on 662. They were placed on monkey on 21st and 22nd.
692	96-120	1		2	2	62	—	
695	96-120	1		2	2	63	—	
689	120-144	2		5	5	67	—	
708	144-168	3		12	12	61	—	
711	168-192	2	1	5	5	54	—	
718	192-216	6	1	12	6	60	—	
694 (c)	216-240	2		3	3	55	—	Blood injected on the 55th day into white rat, with negative result. Blood injected into white rat on 30th day of experiment, with negative result. Incubation period, 10 or 11 days.
782	264-288		3	3	3	49	—	
757	312-336		2	4	4		+	

Thus a dose of arsenphenylglycin of 0.1 grm. per kilogramme will protect a monkey against infection by positive *G. palpalis* if given within 12 days before exposure.

Expt. No.	Period elapsing between the administration of the arsenic and the application of the positive flies.	Number of experiments fed on monkey.		Number of positive flies to which monkey was exposed.		Duration of examination of monkey.	Result.	Remarks.
		First tested on a clean monkey and proved infective.	Not so tested.	1st day.	2nd day.			
	Hours.							
720	72-96	2		4	4	56	—	
719	96-120		3	10	10	61	—	
724	120-144	2	1	5	5	50	—	
774	144-168		4	4	4	65	—	
775	144-168		4	4	3	65	—	
749	168-192	2	2	8	5		+	Incubation period, 10 or 11 days.
748	192-216	1	2	4	1		+	" "

Thus a dose of arsenphenylglycin of 0.05 grm. per kilogramme will protect a monkey from infection by positive *G. palpalis* if given within 7 days of exposure. Using the inoculation method of infection, Mesnil and Kérandel proved that 0.05 of this drug per kilogramme was capable of protecting a monkey from infection up to within 3 days of the administration of the drug.

Conclusions.

I. By feeding a *G. palpalis* which is infective with *T. gambiense* on an animal whose blood contains arsenic administered within 24 to 48 hours, the gut flagellates in the fly may be destroyed. Those of the salivary glands, however, which are the infecting forms, are unaffected. It is highly probable that the gut may become repopulated with flagellates from the salivary glands.

II. Preliminary feeding of flies on arsenic-containing blood has a deterrent effect on the subsequent development of *T. gambiense* ingested within 24 to 48 hours after the arsenic blood.

III. The feeding of flies on arsenic-containing blood immediately after the imbibition of *T. gambiense* usually prevents further development of the trypanosomes in the fly. In the event of development occurring the strain produced is not arsenic-fast.

IV. Arsenphenylglycin exerts a prophylactic effect in a monkey against infection with *T. gambiense* by positive *G. palpalis*; this effect varies with the dosage employed, and is considerably greater than when the trypanosomes are introduced by direct inoculation of infected blood.

8.—THE SLEEPING SICKNESS RESERVOIR ON THE ISLANDS OF LAKE VICTORIA NYANZA.

By H. LYNTHURST DUKE.

(Received October 21, 1912.)

During the last twelve months a considerable amount of attention has been devoted to the part played by wild antelope as a natural reservoir for human trypanosomiasis. The special aspect of the problem studied from this laboratory is that relating to the continued infectivity of the wild flies on the Northern islands of Lake Victoria Nyanza, on many of which situtunga are present in large numbers. A recent visit to the Buvuma group completes a series of investigations involving the numerous islands extending for some 50 to 60 miles along the coast of Uganda, from Sese on the west to Buvuma on the east. The relatively concise experimental conditions obtaining on these islands afford excellent opportunities for unravelling what bids fair to become one of the greatest administrative problems of modern Africa.

The majority of these experiments have already been recorded; as, however, this branch of the work has now been concluded, it will be well to sum up all the available evidence and to test the deductions made.

The Northern islands of the Lake are divided roughly by the natives into three groups. On the west lies the great Sese group, comprising some 50 islands of varying size, and separated by channels ranging from a hundred yards to several miles in width. To the east lies Buvuma; while between the two are a series of islands, the extremes sometimes included in the two great terminal groups, the middle ones formerly independent as regards their native inhabitants. The only species of antelope found on these islands appears to be the situtunga (*Tragelaphus spekei*).

The statement that the bushbuck (*Tragelaphus scriptus*) occurs on Buvuma is probably based upon the marked resemblance between the females of the two species.

Situtunga do not, however, occur upon every island. To judge from native report, which I have been able to verify in several instances, a considerable number of the islands are devoid of these animals, although the natural conditions are apparently favourable. There seems to be no doubt that the normal requirements of this remarkable antelope are papyrus swamps and dense forests; and on the islands at any rate the latter appears to be a condition almost, if not quite, as important as the papyrus. On the mainland, on the other hand, they are often found in large papyrus swamps in districts where there is very little if any forest available. This predilection for forest is an important point in considering the question of this antelope serving as a reservoir for sleeping sickness. For it is well known that *G. palpalis* is not found in papyrus areas pure and simple; and were the species exclusively confined to such localities it might be ignored as a serious factor in fly-borne disease. The southernmost island of the Sese group has from time immemorial been famed as a haunt of the situtunga. At the present time, despite

the periodic massacres which used to be carried out by native hunters, this little island simply teems with buck, although there is not a vestige of papyrus swamp to be seen throughout its coastline. A low rocky island, exposed to the full force of the southern gales, Nkose is thickly forested from end to end right to the water's edge, yet it seems admirably adapted to the requirements of the situtunga.

In several instances, since the removal of the island population, situtunga have migrated across the intervening channels to islands where, according to native report, they were formerly unknown. This would indicate that on inhabited islands the animals were probably liable to extermination by the natives who hunted them unmercifully. The distribution of the other factor in the reservoir problem, *i.e.*, *G. palpalis*, is practically universal throughout the chain of islands. Only the smallest rocks, almost bare of vegetation, are free from fly. On some the fly is present in far greater numbers than on others; thickly forested islands with trees reaching almost to the water's edge constitute the most favourable conditions. From personal experience I have no hesitation in saying that fly are found in large numbers on those islands where situtunga abound. There is one exception, Nkose mentioned above, and this island is peculiar in many ways. Firstly, it was never inhabited by natives though frequently visited by hunters; Secondly, there seems to be no place where the fly could deposit their larvæ. The whole coastline of the island consists either of rock, often rising perpendicularly out of the water, or of narrow beaches of large smooth pebbles without a trace of sand or suitable pupæ ground. Fly are, in consequence, exceedingly rare on this island.

As examples of the coincidence of large numbers of buck and fly on the same island, Damba and Bugalla of the Sese group, and Buziri near Buvuma may be quoted. On all these islands situtunga are very numerous, and this is likewise the case with fly. From the above it is obvious that the conditions on these islands are particularly favourable to the establishment of that frequent interaction between fly and buck which, once given the presence of such an organism as *T. gambiense*, constitutes a reservoir.

A brief résumé may now be given of the experiments already reported from Damba, Bugalla, Kome, and Lwagi islands. I am indebted to Dr. Carpenter for permission to quote his experiments where necessary.

Damba Island.—Hammerton and Bateman found the flies of this island infective to monkeys in several experiments conducted in 1910, and Carpenter, in May, 1911, obtained an infection with 885 flies. In September and October, 1911, blood of situtunga, shot on the fly-ridden S.E. shore of the island, was injected into monkeys and a goat. Two monkeys were employed, each receiving blood from two antelope. Both monkeys and the goat developed *T. gambiense*, the goat also showed *T. vivax* and *T. uniforme*. Thus at least two and possibly all four of the situtunga were infected with *T. gambiense*.

Bugalla.—Early in 1912 Carpenter obtained a *gambiense* infection with some 6,000 flies. Four situtunga were shot here in May, 1912, and their blood injected into two monkeys with negative results. A goat similarly treated became infected with *T. vivax*.

Kome.—Carpenter obtained an infection with 6,000 odd flies late in 1911. No antelope were shot here.

Lwagi.—On this island there are no situtunga, the fauna consisting of hippos, reptiles and birds. In September, 1911, 6,560 flies failed to infect monkeys, and 4,258 were fed on a goat with negative results.

Experiments carried out in August, 1912, on Buziri.—On this island situtunga are present in large numbers and *G. palpalis* abounds throughout its coastline. Blood from 11 situtunga was injected into two monkeys with negative result. Another monkey was infected with *T. gambiense* after being fed upon by 3,060 flies.

In weighing the above evidence it must be borne in mind that when dealing with antelope known to be infected with *T. gambiense*, as is the case with the animals at this laboratory, a considerable number of subinoculations may be performed before a plus result is obtained. This becomes increasingly evident the older the infection. It is thus plain that young animals are more likely to give positive results than adults which in the course of years may have acquired a tolerance to the parasite amounting for practical purposes to complete immunity. In this connection it should be noted that of the four animals shot on Damba three were young; out of the four from Bugalla three were full grown; of the eleven from Buziri only one young individual was obtained, and in this monkey suppuration occurred at the site of injection. It will be seen that the mere numbers of animals investigated is not a fair criterion of the infectivity of the antelope on the island concerned.

The degree of infectivity of the locality is very roughly indicated by the number of flies needed to cause an infection. For the islands considered, the order of infectivity would thus be Damba, Buziri, Bugalla and Kome. Now admitting that the situtunga are acting as a reservoir, the above-mentioned distribution of infectivity might be foretold from a study of the geography of the islands.

On Bugalla the flies were caught on one small strip of shore which was visited fairly frequently by the buck, though the daily presence of Dr. Carpenter's fly boys have now to a great extent driven them elsewhere. The island is a very big one and there are large tracts of forest and open ground inland where situtunga tracks abound and where fly do not occur. The animals can therefore wander far and wide without meeting fly; while the particular piece of shore where the experimental flies were caught is by no means their favourite haunt. The same remarks apply to Kome, where however both buck and fly are much less numerous.

Damba is completely covered with forest except for three or four small clearings overgrown with rank grass. The island is much smaller than Bugalla or Kome, and situtunga are plentiful, frequenting especially the S.E. shore where fly are most numerous. They may be seen here from a canoe morning and evening feeding at lake-edge, far more frequently than on the other islands I have visited. Exposure of the antelope to fly-bite must be very frequent. Buziri is much like Damba as regards its size and the number of situtunga, but the whole centre of the island is clear of forest and dotted with clumps of trees and thickets in which the animals like to lie up. Fly are not so numerous as on that side of Damba where the experiments were conducted, but the antelope must none the less be exposed constantly to their attentions. On this island the fly appear to be distributed very evenly along the whole coast-line. In former days the great majority of the villages were situated close to the water's edge, often immediately behind the papyrus areas. A large part of the coast is occupied by a more or less scanty undergrowth with here and there banana trees and clumps of elephant-grass, and behind these a zone of forest. Even behind the papyrus there is usually a strip of low scrub before the forest is reached; this is often the site of a straggling village and here fly are numerous. The conditions are therefore very favourable to the frequent exposure of the antelope to fly-bite; and doubtless further experiments would give results more nearly approaching those of Damba.

It would thus appear that wherever glossina and buck occur together in numbers on an island, there the fly are found to be infective with *T. gambiense*. The infectivity of the antelope is for many reasons much more difficult to demonstrate; nevertheless the Damba experiments have supplied evidence of the utmost value on this point. Further the degree of infectivity of the fly appears to be directly proportional to the frequency of exposure of the antelope to their bites.

On islands where fly are very numerous and the buck absent the former are apparently non-infective, even although hippos, reptiles and birds abound. This is the case with Kimmi as was shown by Minchin originally and again by Frazer and myself in January and February, 1911. On Nsadzi Island also, where fly are present in one of the bays in considerable numbers and where there are also many hippos, crocodiles and birds, we found the fly non-infective to monkeys in February, 1911. Granted that the numbers tested on these last-named islands are too small to justify the sweeping assertion that the fly are non-infective, none the less plus results have been obtained with far smaller numbers on the islands where antelope are common.

The above facts all point strongly to *Tragelaphus spekei* acting as a true reservoir for *T. gambiense* on these uninhabited islands; and the same doubtless is equally true as regards *T. vivax* and *T. uniforme*.

9.—FURTHER INVESTIGATIONS ON THE RÔLE OF ANTELOPE AS A RESERVOIR OF *T. GAMBIENSE*.

H. LYNDHURST DUKE, B.A., M.B. (Cantab.),

D.T.M. & H. (Cantab.).

(Received November 30, 1912.)

In the Proceedings of the Royal Society, B. vol. 85, pages 299 to 311, experiments were described dealing with the duration of *T. gambiense* in the antelope at Mpumu laboratory, which were infected by the 1908-10 Commission in April and May, 1910. The conclusions drawn from these experiments were (1) that the antelope may remain capable of infecting *G. palpalis* with *T. gambiense* for a period of at least 22 months after their original infection with this trypanosome. (2) That there is some evidence to show that an antelope which has ceased to be infective for *T. gambiense* acquires some degree of immunity against re-infection.

In the following discussion the history of these antelope is followed out from March, 1912, until November, 1912:—

At the commencement of the present investigation the animals were, with one exception, fed upon by infected laboratory-bred *G. palpalis* which had *previously been proved capable of infecting monkeys with T. gambiense*. The exception, Bushbuck 2371, served to some extent as a control to the other animals exposed to re-infection.

Certain experiments were also performed in which the serum of the antelope was investigated for any protective action against *T. gambiense*. Each antelope will be considered separately, together with any experiments conducted with its serum, and a brief reference will be made to its previous history.

Reedbuck 2357.

Original infection by laboratory-bred *G. palpalis* containing a human strain of *T. gambiense*, April, 1910. Latest evidence, previous to this discussion, was obtained in July, 1911, when it proved infective to laboratory-bred flies.

On April 15, 1912, this animal was fed upon by five infected boxes of laboratory-bred *G. palpalis* in which were a total of 14 flies showing infected salivary glands.

On April 16 and 17, 1912, it was fed upon by four infected boxes containing a total of four flies with infected salivary glands.

After allowing sufficient time for trypanosomes to appear in its blood, this reedbuck was tested by clean laboratory-bred flies and by injection of its blood into susceptible animals.

The results were as follows:—

Fly Experiments.

Expt.	Period for which flies fed upon antelope.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected.	Containing flagellates.			
752	1912. May 22-23	31	27	31	0	38	—	

Injection Experiments.

Date.	Expt. No.	Quantity of blood injected.		Result.	Animal used for injection.	Incubation period in days.
		Citrated.	Pure.			
29.4.12	728		2½ c.c.	+	Monkey	15
31.5.12	760		1½ c.c.		Monkey ran away before trypanosomes could appear.	
13.7.12	792		3½ c.c.	—	Monkey.	
21.8.12	838	5 c.c.		—	"	
21.8.12	839	7 c.c.		—	"	

Owing to the animal becoming savage and fighting with the other antelope in the large house, it had to be removed to a fly-proof cattle kraal some one-and-a-half miles away. Here in very cramped quarters it refused to eat satisfactorily and died during a heavy storm, apparently from trauma, direct or otherwise. Experiments 838 and 839 were performed a few hours after death. A careful microscopic examination of the liver, spleen, and lung by Miss Robertson failed to reveal any sign of trypanosomiasis.

This animal, therefore, after giving no signs of infection for 8 months, responded to re-infection by positive flies. The infection was, however, apparently a transitory one, as three inoculation experiments performed in the third and fourth months after re-infection failed to show the presence of *T. gambiense*. It will be remembered that after the original infection of all these antelope in 1910 plenty of evidence was available as to the presence of trypanosomes in their blood during the early stages of the disease.

Reedbuck 2431.

Original infection by laboratory-bred flies in May, 1910. Latest evidence, previous to this discussion, of the infectivity of this animal was obtained in November, 1910.

Attempts were made to re-infect this animal as follows:—

March 26 and 27, 1912, fed on by one infected box containing two flies with positive salivary glands.

2431 subcutaneously, the mixture having stood at N.T.P. for 15 minutes. Trypanosomes appeared in the rat after an incubation period of 6 days.

Experiment 885 (White Rat).—On 1.10.12 received 20 m. serum of Reedbuck 2431, and on 2.10.12 received 2 m. of citrated blood of Monkey 728 (+ +). Trypanosomes appeared in the rat after 6 days incubation period.

Experiment 887 of the above injection table serves as a control to the serum of Reedbuck 2431 during these experiments. The serum of this animal therefore shows no sign of any protective action against *T. gambiense*.

This animal limps badly from injuries received in handling for experimental purposes. It has lost flesh slightly, but still appears in good general health.

Reedbuck 2359.

Original infection by laboratory-bred flies, April, 1910. Latest evidence of the infectivity prior to this paper obtained in July, 1911. Some experiments, conducted in June, 1911, failed to reveal any sign of protective action against *T. gambiense* in the serum of this reedbuck.

In April, 1912, this animal was fed upon by infected boxes of laboratory-bred *G. palpalis* as follows:—

8.4.12, two boxes containing three flies with infected glands.

9 & 10.4.12, three boxes containing five flies with infected glands.

11.4.12, one box containing two flies with infected glands.

The following experiments were then performed:—

Fly Experiments.

Experiment No.	Period for which flies fed on antelope.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected.	Containing flagellates.			
742	1912. May 21-27	63	46	63	2	38	—	Monkey remained negative. The glands of these flies were not dissected out.
743	„	33	21	33	0			
	Totals ...	96	67	96	2			

Injection Experiments.

Date.	Experi- ment No.	Quantity of blood injected.		Result.	Animal used for injection.	Incubation period in days.
		Citrated.	Pure.			
27.4.12	725		1	—	Monkey.	8
1.5.12	736		1	+	"	
3.6.12	767		1	—	"	
13.7.12	794		2	+	"	12
20.9.12	876		2½	—	"	

Thus after 8 months, during which it gave no sign of infectivity, this antelope was susceptible to re-infection by positive *G. palpalis*. This second infection appeared to be less severe than the original one and also showed some signs of being of short duration.

The following experiments were devised to see whether the serum of this animal has acquired any action against *T. gambiense*. The trypanosome-containing blood selected was that of 728, which was inoculated from Reedbuck 2357 (see *supra*). On the day of the experiments this monkey was showing trypanosomes in its blood though in small numbers.

Experiment 872 (White Rat).—On 20.9.12 received 1 m. citrated blood of Monkey 728 subcutaneously. The rat developed trypanosomes in its blood after an incubation period of 11 days.

Experiment 873 (White Rat).—On 20.9.12 received subcutaneously a mixture of 1 m. citrated blood of Monkey 728 and 10 m. serum of Reedbuck 2359, the mixture being injected immediately. This rat never developed trypanosomes though under daily observation for 45 days.

Experiment 874 (White Rat).—On 20.9.12 received 8 m. serum of Reedbuck 2359 on one side of the body and 1 m. citrated blood of Monkey 728 on the opposite side simultaneously. The rat developed trypanosomes after 10 days incubation period.

Experiment 875 (White Rat).—On 20.9.12 received 20 m. serum of Reedbuck 2359, and on 21.9.12 received 1 m. of citrated blood of Monkey 728 (+). Trypanosomes developed after 7 days incubation period.

The feature to be noticed in the above experiments is the long incubation period in both the rats inoculated with blood taken from Monkey 728 on 20.9.12. The very small quantity of trypanosomes was inoculated in each case, and it is very doubtful whether the single negative result of Experiment 873 has any significance. For the rest no sign of protective action was noted. Experiment 876 of the injection table of this antelope serves to control the serum at the time of these experiments.

This animal was injured sometime ago while being caught for experimental purposes. It shows signs of joint lesion at fetlock and shoulder and limps badly.

Bushbuck 2371.

Original infection April, 1910. Latest evidence of infectivity prior to this paper occurred in February, 1912.

No attempt was made to reinfect this antelope in order that it might act as a control to the other experiments.

The following experiments were carried out.

Fly Experiments.

Experiment No.	Period for which flies fed on the antelope.	Number of flies.				Length of experiment in days.	Result on clean monkey.	Remarks.
		1st day.	30th day.	Dissected.	Containing flagellates.			
770	1912. June 4-9	83		83	0	28		Fed on fowl throughout the experiment. "
772	June 6-10	39		39	0	27		
	Totals ...	122		122	0			

Injection Experiments.

Date.	Experiment No.	Quantity of blood inoculated.		Result.	Animal used for injection.	Incubation period in days.
		Citrated.	Pure.			
31.5.12	759		1½ c.c.	—	Monkey.	
13.7.12	795		3	—	"	

This animal has shown no evidence of infectivity since February, 1912, *i.e.*, for eight months. It must be recollected, however, that previous to this date a period of nine months had elapsed without any positive evidence. The animal is in excellent condition at the date of writing.

Waterbuck 2378.

Original infection April, 1910, latest evidence of infectivity obtained in August, 1910. Fed upon by infected *G. palpalis* on August 10th to 12th, 1911, after which it gave however no evidence of reinfection.

Injection Experiments.

Date.	Experiment No.	Quantity of blood injected.		Result.	Animal used for injection.	Incubation in days.
		Citrated.	Pure.			
30.7.12	812		7	—	Monkey.	
30.7.12	811		20 m.	—	White rat.	

The following experiments were performed with the serum of this animal.

Experiment 809 (White Rat).—On 30.7.12 received 2 m. citrated blood of Monkey 794 (*T. gambiense* ++) subcutaneously. Trypanosomes appeared in the rat after an incubation period of 8 days.

Experiment 808 (White Rat).—On 30.7.12 received subcutaneously a mixture of 8 m. serum of waterbuck, 2 m. citrated blood of monkey 794. Trypanosomes appeared after 7 days.

Experiment 813 (White Rat).—On 30.7.12 received subcutaneously a mixture of 8 m. serum of waterbuck and 2 m. citrated blood of Monkey 794, the mixture having stood for 2 hours. Trypanosomes appeared after 7 days' incubation period.

Experiment 810 (White Rat).—On 30.7.12 received subcutaneously 20 m. serum of waterbuck. On 31.7.12 received 2 m. citrated blood of Monkey 794 (++) subcutaneously. Trypanosomes appeared after 5 days.

Experiments 811 and 812 of the above table served as controls of the serum used in these experiments.

This waterbuck, therefore, has apparently acquired a degree of passive immunity to *T. gambiense* sufficient to enable it to resist re-infection by positive *G. palpalis*. Its serum has, however, no protective action against this trypanosome.

The animal is in excellent health at the time of writing.

Bushbuck 2328.

Originally infected, April, 1910. Latest evidence of infectivity prior to this discussion, August, 1910. An attempt was made to re-infect this animal by feeding infective *G. palpalis* upon it from June 3 to 10, 1911. This apparently failed. Some experiments conducted with the serum of this animal in July, 1911, also failed to reveal any protective action against *T. gambiense*.

On 16.4.12 it was fed upon by two infective boxes containing a total of four flies with positive salivary glands.

On 17.4.12 it was fed upon by three infected boxes containing in all seven flies with positive glands.

On 18, 19, and 20.4.12 it was fed upon by two infected boxes containing four flies with positive glands.

The following experiments were carried out:—

Fly Experiments.

Experiment No.	Period for which flies fed on antelope.	Number of flies.				Length of experiment in days.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected.	Containing flagellates.			
744	1912. May 21-25	68	52	68	0	28		Fed on cock throughout experiment.
745	" 21-25	38	30	38	0	28		
755	" 27-	56	52	56	0	22		
756	June 1.							
	" 29- June 1.	88	86	88	0	23		
	Totals ...	250	220	250	0			

Injection Experiments.

Date.	Experiment No.	Quantity of blood inoculated.		Result.	Animal used for inoculation.	Incubation in days.
		Citrated.	Pure.			
29.4.12	729		1	—	Monkey.	
3.6.12	768		1	—	"	

The animal thus resisted a second and more severe attempt at re-infection some 9 months after the previous attempt.

On 4.9.12 Bushbuck 2328 was inoculated subcutaneously with 2 cc. citrated blood of Monkey 728 (*T. gambiense* +), and subsequently the following injections were made.

Date.	Experiment No.	Quantity of blood inoculated.		Result on clean monkey.	Animal used for injection.	Incubation period.
		Citrated.	Pure.			
11.9.12	860		2	—	Monkey.	
1.10.12	884		2½	—	"	
12.10.12	897		1½	—	"	

This bushbuck has thus resisted all attempts at re-infection, whether by the agency of positive flies or by direct inoculation. It would thus appear to have acquired immunity against *T. gambiense*.

The following serum experiments were carried out:—

Experiment 784 (White Rat).—On 27.6.12 received subcutaneously 2 m. citrated blood of Monkey 751 (*T. gambiense* + + +) after standing at N.T.P. for 2 hours. Trypanosomes appeared in the rat after 5 days.

Experiment 783 (White Rat).—On 27.6.12 received subcutaneously a mixture of 2 m. citrated blood of Monkey 751 and

20 m. serum of Bushbuck 2328 after standing at N.T.P. for 2 hours. Trypanosomes appeared in the rat after 6 days.

Experiment 785 (White Rat).—On 27.6.12 received simultaneously 2 m. of citrated blood of Monkey 751 (+ + +) on one side of the body and 20 m. serum of Bushbuck 2328 on the opposite side. Trypanosomes appeared in the rat after 5 days.

The serum of this antelope appears therefore to exert no protective influence against *T. gambiense*. The animal is at present in excellent condition.

It may be noted that the trypanosomes employed for testing the protective action of the sera of the above animals were derived in each case from one or other of the laboratory antelope after long residence in these animals, either by direct inoculation into monkeys or, as in the case of Monkey 751 carried by laboratory-bred *G. palpalis* from antelope to monkey (box 709, reedbuck 2431).

Reviewing the above experiments it will be seen that of the five animals exposed to re-infection two showed complete resistance, while the other three developed more or less transitory infections. On no occasion has any protective action been observed in the sera of these animals, with the doubtful exception of Experiment 873 of Reedbuck 2359. In this last the minute quantity of trypanosomes employed, together with the very considerable dilution with serum before injection, may have vitiated the experiment.

It would appear that these laboratory antelope are acquiring a degree of passive immunity against *T. gambiense* which will probably become strengthened by each successive attempt at re-infection. There is no reason to conclude that any difference exists between the various species investigated as regards their susceptibility to the trypanosome, as Bushbuck 2371 and 2328 illustrate respectively the longest and shortest duration of infectivity. This Bushbuck 2371 was proved to be infected some 22 months after the original infection, while Bushbuck 2328 has remained negative to all tests ever since the 4th month after its original infection.

It appears that individual antelope vary in their reaction to *T. gambiense*, but all agree in the absence of any active immunity against this organism.

As regards the general significance of the facts brought out in these antelope experiments it is hardly possible at present to pronounce a final opinion. There is no doubt that antelope may and will act as a reservoir to the human trypanosome of Uganda, but how soon they would acquire immunity, when subject to constant re-infection by fly in nature cannot be decided.

Individual idiosyncrasies will play an important part together with the frequency of the exposure to infection. Immunity, when once acquired, may last at least 26 months, as is the case with Bushbuck 2328. On the other hand, it is impossible to prophecy the behaviour of Reedbuck 2431 and 2359 in this respect even if they do eventually acquire immunity,

Mpumu,

November 3, 1912.

10.—A TRYPANOSOME FROM BRITISH EAST AFRICA SHOWING POSTERIOR-NUCLEAR FORMS.

By H. LYNTHURST DUKE, B.A., M.B., Cantab., D. T. M. & H.,
Cantab. (With a Note on its Developmental Stages in
Glossina palpalis. By MURIEL ROBERTSON, M.A.)

(Received November 30th, 1912.)

The trypanosome which forms the subject of this paper was forwarded to Mpumu by Mr. E. Montgomery, Veterinary Pathologist to the British East African Government. The organism was obtained originally from a donkey which was supposed to have contracted the disease at a spot on the *safari* road from Ngobotok to Baringo, about one day's march from the former, on the Wewe River. This place is apparently known to the natives as being very dangerous for cattle, although the grazing is good. On this account they only water their animals at night, grazing them during the day on higher land. Baringo lies approximately 0.30 N. and 36.10 E., about 70 miles north of Nakuru. Dr. Ross, of Nairobi, writes that on the stretch of road above mentioned, tsetse are known to occur at three places. The species found are—*G. pallidipes* and *G. longipennis*, while just north of Baringo *Glossina brevipalpis* occurs.

Several other donkeys were infected on the same journey, and the disease is described by the local veterinary officer, Mr. A. T. Crowther, as lasting four days.

I have to express my indebtedness to Mr. Montgomery for his kindness in supplying me with the charts of the animals inoculated at his laboratory and other useful information, and also for the loan of important literature bearing on the question of diagnosis.

The various experiments detailed below were devised with a view to deciding, if possible, the identity of the organism. Some very interesting facts have come out in the process which throw a side-light of some importance on one of the most recent problems of trypanosomiasis, namely, the significance of posterior-nuclear forms in species diagnosis.

The morphology of the trypanosome will be considered first and subsequently its animal reactions, behaviour with human serum, and transmission by laboratory-bred *G. palpalis*.

Morphology.

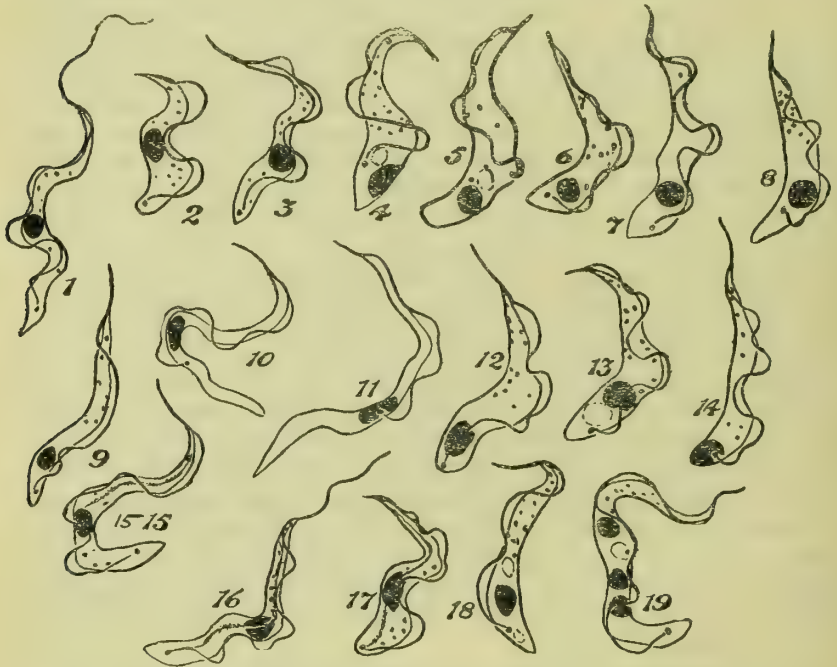
(a) Living, unstained.

A feature of the movement of this trypanosome is the ceaseless wriggling of the body with very little resulting translation through the field. It is common to see individuals more or less passive posteriorly with the flagellar end in very active motion, and, as it were, probing the neighbouring corpuscles. Translatory movements, when they occur, are of a steady gliding character with very little jerking of the body axis.

(b) Fixed and stained.

Protoplasm.—Generally shows a varying number of granules similar to those figured by the 1908-10 Commission for *T. brucei*. These may be seen distributed throughout the body or may be confined either to the portion posterior or anterior to the trophonucleus; they occur, however, more frequently in the latter situation.

Kinetonucleus.—There is nothing remarkable about the shape or size of this structure. It may rarely be seen at the extreme posterior end, more commonly a more or less pointed snout of protoplasm projects beyond it posteriorly. As is shown in the text-figures (4-5, 10-11) the kinetonucleus may on rare occasions be found unusually far forward both in relation to the posterior end of the body and to the trophonucleus. A condition similar to that described for *T. rhodesiense* is thus obtained.



Text Fig.—Forms from the peripheral blood.

1-3 normal types (1 long, 2 short, 3 intermediate), from monkey 821; 4-8 from dog 807; 9-11 from monkey 830; 13 from rat 833; 14 and 18 from rat 832; 15-17 from monkey 821; the specimens show the volutin line; 19 aberrant form showing signs of multiple division.

Magnification = 2,000 diameters.

Trophonucleus.—In a very considerable number of cases it is situated in the posterior third or fourth of the body, often close to the posterior end. This posterior arrangement of the nucleus is very much more frequent in the short and intermediate types than in the long forms. The proportion of posterior-nuclear types varies in different host animals; it is relatively common in dogs and rats, but is rarely found in monkeys. Occasionally these

forms may amount to as much as 30 per cent. of the parasites present. (Cf. text-figures 4-9 and 12-14.)

Undulating Membrane.—This is well marked.

Flagellum.—The long forms show a long free portion to the flagellum. The stumpy types usually have a short free portion, but in some the protoplasm extends to the tip of the flagellum. In certain specimens a clear vacuole, measuring on occasions 3 microns in diameter, is seen just anterior to the kinetonucleus.

On one occasion an individual was seen in the peripheral blood of a dog, which suggests (see Fig. 19) a process of multiple division. This is a remarkable exception from the general rule of binary fission prevalent among mammalian trypanosomes generally, with the exception of *T. lewisi*. A similar instance is recorded by Hindle¹ for *T. dimorphon*.

In a certain proportion of the specimens a staining line may be seen (Figs. 15-17) passing from the kinetonucleus to the trophonucleus, and thence to the anterior end of the body. In Giemsa preparations this structure strains from a violet-blue to a purple-red; it may in some cases extend only from the kinetonucleus to the trophonucleus, or only from the trophonucleus to the anterior end of the body. This portion of the line (i.e., between the trophonucleus and the anterior end of the body) may sometimes show bead-like thickening, and may also on occasions be thrown into spiral coils; this is, however, not a very frequent occurrence. In wet preparations stained by Heidenhain's method the line is also visible and shows the same relations as in the Giesma films; it must, however, be observed that the process of differentiation may easily be carried too far to show either the line or the meta-chromic granules which are so striking a feature of the Giemsa results. The structure here noted is clearly similar to the line described by various observers² in the case of *T. brucei*; Swellengrebel³ considers it to be composed of volutin. No experiments were conducted to determine the composition of the line in the present case.

The striking feature in the above description is of course the occurrence of the posterior-nuclear type of trypanosome. This curious condition has of late been considered peculiar to *T. rhodesiense* and regarded as a phenomenon of great diagnostic value. In the *Sleeping Sickness Bulletin* 39 Dr. Wenyon's discovery of similar forms in slides of *T. pecaudi*, taken by him in the Soudan, is mentioned. He does not seem convinced as to the exact identity of this trypanosome, as he remarks that if it should prove to be *T. brucei* the value of the nuclear arrangement as a distinguishing feature of specific importance is further reduced. Bruce (*Sleeping Sickness Bulletin* 39) points out that did such forms occur in *T. brucei* they would have been described long since, as this species has been more continually under observation than perhaps any other pathogenic trypanosome.

(¹) Hindle, E., Univ. Calif. Pub., Zool., Vol. 6, No. 6, pp. 127-144 (1909).

(²) Robertson, Proc. Roy. Phys., Edin. 1906.

(³) Swellengrebel, C. R. Soc. Biol. Vol. 64. 1908. p. 38.

It is interesting to note that in forwarding the present trypanosome to Mpumu in the first instance, Mr. Montgomery mentioned that it was supposed to be *T. pecaudi*. In the event of Wenyon's diagnosis of *T. pecaudi* being correct, the occurrence of the posterior nucleus in this species and in the one under discussion increases the probability of their being identical.

As will be seen below, the measurements vary from 12 microns to 38 microns, the species is markedly "dimorphic" in the sense that long and short forms are found in the same slide. Bouffard¹ finds forms reaching 40 microns in length, and draws attention to the great breadth of the stumpy type which may reach 4 microns. Cazalbou² and Laveran³ give 14 to 20 microns and 25 to 35 microns for the short and long types respectively. From the point of view of measurement, therefore, there is nothing in Table I. to exclude *T. pecaudi*. Again, in connection with the table of animal reactions it will be seen that there is a certain amount of correspondence between the *T. pecaudi* of Bouffard, Laveran, and Cazalbou, and this trypanosome. The question of diagnosis will, however, be dealt with later on, when it will be seen that, either the posterior-nuclear type of trypanosome is not pathognomonic for *T. rhodesiense*, or else the reactions of this trypanosome may vary greatly under different natural conditions.

Table I. represents measurements of the trypanosomes from various experimental animals on ten different occasions. The slides were fixed by exposure to osmic vapour and immediate immersion in alcohol absolute; they were thereafter stained by Giemsa's method. One hundred consecutive examples were drawn from each preparation, dividing forms being passed over.

The examples given in this table were selected with a view to illustrating the great variation in length shown by this species. Slides were made on days when the trypanosomes were fairly numerous, and an effort was made to obtain representatives of each variation of type. It will be seen that the extreme measurements are 13 microns and 38 microns, though in the course of an extensive series of measurements from Monkey 821, Miss Robertson found three examples of 12 microns in length. In these experiments it was also found that the individual variation in the monkey extended from 12 microns to 37 microns, while the daily average of 100 specimens may vary between 16.4 and 26.8 microns. It is thus obvious that no matter how great be the number of trypanosomes measured the conclusions are valueless unless some systematic method is followed. For similar reasons a curve constructed from the 1,000 individuals of Table I. will give an entirely false impression as to the morphology of the species.

Stephens and Fantham in their measurement of *T. rhodesiense* summarised in *Sleeping Sickness Bulletin* 39 have at length realised the futility of the haphazard methods of measurement at present in vogue. They lay stress upon the importance of drawing a number of trypanosomes daily, attention being directed to

(¹) Bouffard, Annal. Inst. Pasteur. Jan. 1908.

(²) Cazalbou, Rev. Gen. de Méd. Vétér. Mai, 1907.

(³) Laveran, Annal. Inst. Pasteur. Mai, 1907.

Table I.

Length in μ .	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.	Average.
Monkey 822. 7th day of infection ...			1	1			1	3	1	3	1	3	2	2	4	4	6	8	12	6	14	12	8	4	1	3	30.37
Dog 807. 6th day of infection ...			1	5	2	4	7	1	3	2	8	15	7	8	7	9	10	8	1	2							24.54
Rat 833. 5th day of infection ...			3	4	6	13	13	10	13	10	6	7	5	2	4	4											20.88
Rat 832. 12th day of infection ...		1	4	7	24	13	15	15	14	4	2			1													18.67
Rat 832. 5th day of infection ...		1	3	10	11	18	21	10	11	6	4	2	1	1			1										19.12
Dog 835. 6th day of infection ...						1	2		3		5	5	4	4	3	4	17	14	12	19	6	1					28.76
Dog 807. 14th day of infection ...	2	5	6	14	14	15	10	8	9	12	2	2		1													18.41
Monkey 821. 13th day of infection ...		1	11	17	17	8	12	10	6	6	3	2	3	1	1	1			1								18.74
Monkey 821. 19th day of infection ...		13	23	28	14	5	8	4	2	1		1		1													16.49
Monkey 821. 7th day of infection ...			1	3	1	4	2	2	3	5	4	6	5	6	8	9	7	8	14	8	1	1		1	1		26.61

the day of infection and the host from which the trypanosomes are taken. These points are certainly important, but until the governing principle is properly understood their realization will serve but little purpose. In my opinion the key to the question lies in Miss Robertson's discovery of the endogenous cycle of development in the peripheral blood of the mammalian host, recently published in connection with *T. gambiense*, and still more recently confirmed by a long series of observations with the subject of this paper. In this connection it will be well to test the conclusions of Fantham and Stephens by comparing measurements of, say, a 100 specimens of this species measured on the same day of the infection.

1. In different species of susceptible animals ... Table A

2. In different individuals of the same species
of susceptible animal Table B

The evidence obtained will, I think, show immediately that there is some important factor at work other than those suggested by the Liverpool workers.

Table A.

Animal.			Day of infection.	Average of 100 trypanosomes.	Difference between the two averages.
				μ .	μ .
Dog 807	...	...	6th	24.54	2.21
Monkey 821	...	...		26.75	
Dog 835	...	...	6th	28.76	2.01
Monkey 821	...	...		26.75	
Rat 832	...	...	12th	18.67	2.9
Monkey 821	...	...		21.5	
Rat 833	...	...	12th	21.80	.29
Monkey 821	...	...		21.51	
Dog 807	...	...	14th	18.41	.0
Monkey 821	...	...		18.4	

Table B.

Rat 832	...	...	5th	19.12	1.76
" 833	...	...		20.88	
Dog 807	...	...	6th	24.54	4.22
" 835	...	...		28.76	
Monkey 821	...	...	7th	26.61	3.76
" 822	...	...		30.37	
Rat 832	...	...	12th	18.67	3.13
" 833	...	...		21.80	

The most that can be said from a consideration of these results is that for any species of trypanosome there is a certain general correspondence between the day of an infection and the type of trypanosome predominating in the peripheral blood, *i.e.*, short, long, or intermediate forms. The degree and constancy of the coincidence varies within such wide limits that it cannot be relied

upon even in the earlier days of the infection, when the idiosyncrasies of the host animal have presumably but little influence on the morphology of the organisms.

It will, I think, be granted that the experimental conditions of Table A are less concise than those of Table B, and that coincidence is the more likely to explain the results. In Table B, however, where short of an appreciation of the cycle idea the conditions seem ideally definite, the discrepancies are the more obvious.

In one case where 100 trypanosomes were drawn from each of the two dogs 807 and 835 on the 6th day of the infection, there is difference of 4.22 microns between the resulting averages, while the apex of the two corresponding curves would be at 24 microns and 32 microns respectively.

Miss Robertson has now shown the endogenous cycle to occur in the two species investigated up to the present, and there seems no reason to doubt that confirmation will shortly be forthcoming from workers at home. At present the biometric method as applied to trypanosomes is admittedly unsatisfactory in its results, and this can be well understood if there is for each species of trypanosome a definite cycle of development in the vertebrate blood from short through intermediate to long forms and so on indefinitely. To obtain a proper appreciation of the length variation of any trypanosome species, it will thus be necessary to make daily measurements of, say, 100 specimens throughout a complete revolution of the cycle as signalled by the reappearance of the long form of trypanosome again. On this theory the discrepancies exposed above between results obtained on the same day of the infection in the same species of host may be explained by the cycle in the different individual animals, and this probably becomes more marked later in an infection. From a diagnostic point of view, what is required is a curve representing the possible range of form for the species concerned, and this will be obtained from a consideration of a complete cycle revolution in the peripheral blood of the vertebrate.

Animal Reactions.

Table II.

Animal.	Source of virus.	Incubation in days.	Duration of disease.	Remarks.
Monkey 821	Dog 807 ...	5	days. 39	In good condition after 67 days. In good condition after 69 days.
" 857	Sheep 847...	10?		
" 853	Dog 835 ...	6		
" 848	Monkey 822	10	44	Received 3½ c.c. human serum and corpuscular debris on 58th day of infection. Slight loss of flesh after 80 days. <i>cf.</i> Table III.
" 849	" 822	5	45-	
" 822	Dog 807 ...	5		

Animal.	Source of virus.	Incubation in days.	Duration of disease.	Remarks.
Monkey 830	Infected <i>G. palpalis</i> .	?	days. 32-	Received 3½ c.c. human serum on 12th day of infection. <i>cf.</i> Table III.
" 831	"	?		Received 3½ c.c. human serum on 21st day of infection. Emaciated and weak after 43 days. <i>cf.</i> Table III.
" 868	"	?		Received 3½ c.c. human serum on 7th day of infection. Lively with no objective symptoms after 28 days. <i>cf.</i> Table III.
" 869	"	?		Lively with no objective symptoms after 38 days.
" 877	"	?		Slight emaciation after 25 days.
" 878	"	?	15	Emaciation marked.
" 879	"	?		Some emaciation after 26 days.
" 880	"	?	21	Emaciation marked.
" 882	"	?		Emaciated, weak and somnolent, after 29 days.
" 883	"	?		Some emaciation after 23 days.
" 891	"	?		Weak. [Now dead, after 26 days.]
" 894	"	?		In good condition after 24 days.
*Dog 807 ...	Donkey 32	?	15-	Marked emaciation and weakness after 26 days.
" 835 ...	Dog 807 ...	5	26	Very marked emaciation : conjunctivitis.
Rat (White) 832.	" 807 ...	4	27	On inoculation a vigorous healthy dog. Corneal opacity very marked during last 10 days.
" 833	" 807 ...	4	28	10 m. blood citrate used.
" 846	Goat 834 ...	7	19	3 m. " "
" 865	Monkey 857	5	36	Received " 20 m. " human serum on 24th day of infection <i>cf.</i> Table.
" 866	" 857	9	30	Received 20 m. human serum on 14th day of infection and 20 m. guinea pig serum on 24th day. <i>cf.</i> Table.
" 888	" 830	11		Trypanosomes at inoculation mixed with human serum. In excellent condition after 32 days.
" 889	" 830	7	21	2½ m. blood citrate used. Control to 888.
" 895	" 831	4	22	5 m. blood citrate used. (Very emaciated : conjunctivitis.)
" 896	" 831	8		Trypanosomes at inoculation mixed with human serum. 5 m. blood citrate used. In excellent condition after 22 days.
Goat 834 ...	Dog 807 ...	5		Shews some emaciation and general weakness after 73 days. No oedema.
Sheep 847 ...	" 835 ...	8		Appears in excellent health after 65 days.
*Ox 114 ...	Donkey 32	?		Temperature rose 5-10th day and again 13-14 days. Appears to be losing condition : shews no other symptoms. Proved infected by sub-inoculation.

were insusceptible to this trypanosome, an experiment was performed to see whether they could be infected by the bite of positive *G. palpalis*. Box 859, which had a few days before infected a monkey, was placed for two days on a clean guinea-pig 898. The flies were subsequently dissected, and fly number 40 of Table V. was found. This fly had guinea-pig's blood in it, and had obviously fed well on at least one of the two days.

The guinea-pig was examined daily until the date of writing, and on no occasion were trypanosomes seen in its blood, *i.e.*, for 19 days.

Another interesting point noted by Bouffard is that in certain cases monkeys recovered from an infection with *T. pecaudi*. Sufficient time has not yet elapsed to form an opinion on this point in the present instance.

The difference in the length of incubation period in Experiments 865 and 866 of Table II. is interesting. Both animals were carefully examined in the same manner; nevertheless trypanosomes did not appear in rat 866 until the 4th day after their appearance in 865. It is to be noted also that after their first appearance in 865 none were seen in the peripheral blood until the 9th day, and a similar occurrence was observed with rat 889, where trypanosomes first showed on the 7th day in very small numbers and then disappeared until the 11th day. In the two experiments given below, in which the protective action of human serum is investigated, trypanosomes behaved in a similar manner in the serum animal, appearing in small numbers and then disappearing, but in both cases they were absent from the blood for a very considerable time.

Of the three observers cited above, neither Laveran nor Cazalbou mention anything peculiar about the incubation period of their *T. pecaudi* in guinea-pigs. Laveran gives the average duration of the disease in these animals as 40 days, though some of the animals died at 18 and 23 days. There is thus a considerable difference between the trypanosomes of Bouffard and Laveran in this respect. It is, however, unwise to lay too much stress on the behaviour of guinea-pigs in distinguishing between two species of trypanosomes. This is well shown in the case of *T. pecorum*, which, though at first considered not to be inoculable into guinea-pigs, afterwards affected these animals. As far as animal reactions are concerned, it may be said that the general behaviour of the trypanosome of this paper corresponds roughly with that of *T. pecaudi*, the exceptions being the relative insusceptibility of guinea-pigs and the donkey reaction. In consideration of evidence to be given later, it may be mentioned in passing that the *T. pecaudi* of Bouffard shows greater resemblance to the present trypanosome than the *T. pecaudi* of either Laveran or Roubaud and Bouet. Other important trypanosomes to be considered are *T. brucei* and *T. rhodesiense*. A mild strain of nagana would give results similar to those of the Table II., with the exception perhaps of the guinea-pigs. *T. brucei* is known to cause a chronic disease in these animals. The lack of objective symptoms in sheep and goats appears to exclude *T. rhodesiense*, as indeed does the general scale of virulence.

Action of Human Serum.

The following experiments were devised to test the susceptibility of this trypanosome to human serum:—

Table III.

Animal treated.	Ultimate source of virus.	Quantity of human serum inoculated.	Native supplying serum.	Condition of blood at time of inoculation.	Hours after inoculation of serum.											Remarks.	
					16.	20.	25.	41.	65.	89.	113.	137.	161.	185.	209.		233.
Monkey 830.	Infected <i>G. palpalis</i> from dog 807.	3½ c.c.	E	+++	+++	+++	+++	-	+	+	++	+++					
" 868	Infected <i>G. palpalis</i> from monkey 822.	"	M	++ First day T's seen in blood.	++	+	++	-	-	-	-	-	+				
" 831	Infected <i>G. palpalis</i> from dog 807.	"	M	+++	+	+	+	+	-	+	+	+	+				
" 822	Dog 807 ..	"	M	++	+	+	-	-	-	-	-	-	-	-	+	++	A considerable amount of corpuscular debris was mixed with the serum here.
" 848	Monkey 822.	"	M	++	+	+	+	+	+	++	-	+	+				
" 849	" "	Control same conditions as 848.		+	+++	+++	+++	+++									
	20 m.	as 848.															
Rat 866..	Monkey 857.		E	+++	++	++	++	-	+	+	+++	+++					
" 865..	"	"	M	++++	+++	+++	++	+	+	++	+++						

In the case of the rats 866 and 865, control animals under identical conditions of infection showed numerous trypanosomes on each day of examination during the period, without any drop such as is seen in the serum animals 41 hours after inoculation.

Experiments *in vitro* with this trypanosome and human serum failed to reveal any susceptibility over controls performed with ordinary citrate solution. An experiment was performed to test the action of guinea-pig serum. *In vitro* the trypanosomes mixed with the guinea-pig serum showed a greater mortality after five hours than the untreated controls.

A rat showing this trypanosome in very large numbers in the peripheral blood was inoculated with 20 m. serum of clean guinea-pig. The course of the infection was apparently uninfluenced in any way, parasites being present in undiminished numbers in the peripheral circulation.

The following experiments were devised to test the protective action of human serum against this trypanosome when injected into rats. In each case the control rat received a small quantity of citrated blood swarming with trypanosomes, while the other was inoculated with a mixture of the same quantity of the blood-citrate and 30 m. of human serum. The mixture was allowed to

stand some 5 to 10 minutes before inoculation, as also was the control blood. The results were as follows:—

Experiment 1.

Control rat, incubation period 7 days.

Serum rat, incubation period 11 days.

(Amount of blood citrate used $2\frac{1}{2}$ m.)

The serum rat (888) of this experiment showed trypanosomes on the day after their first appearance. Since then, though examined daily, no trypanosomes have been seen, while the rat remains lively.

Experiment 2.

Control rat, incubation period 4 days.

Serum rat, incubation period 8 days.

(Amount of blood citrate used 5 m.)

The serum animal of this experiment (896) showed trypanosomes for a single day only. Since then, though examined daily, no trypanosomes have been seen in peripheral blood, and animal remains well.

From the above it will be seen that this trypanosome is susceptible to the action of human serum both curatively and protectively. The curative action is, however, apparently weak and uncertain, and it must be borne in mind that in a discussion (Proceedings of the Royal Society, B. vol. 85, p. 168, 1912) it was pointed out that human serum had quite a marked effect on *T. gambiense* in monkeys, though showing none in rats. A control experiment performed simultaneously with those of Table III. of this paper, in which the serum of native M. was injected into a monkey suffering from an undoubted human strain of *T. gambiense*, trypanosomes disappeared from the blood for 7 days after inoculation. The protective action is considerably more marked, though complete prevention of infection was not observed. It is not possible to state at present whether the course of the disease is affected in the serum animals, though the behaviour of rats and monkeys would indicate that the curative action is slight. Guinea-pig serum exerts no curative effect, nor does admixture with this serum cause any agglutination *in vitro*. According to Laveran, guinea-pig serum causes marked agglutination of *T. brucei in vitro*,¹ which also reacts readily to human serum. It is not easy to gather the significance of these serum experiments in forming an opinion as to the identity of the present trypanosomes. *T. brucei* and *T. rhodesiense* are known to be susceptible to human serum, particularly *T. brucei*. The reaction of *T. rhodesiense* may vary considerably in degree. I have no information as to the behaviour of *T. pecaui* in this respect, though, *a priori*, it may be supposed to be susceptible. On the whole, it would appear that this section of the evidence tells rather against *T. brucei*, but the ease with which strains of *T. rhodesiense* may become resistant to human serum makes comparison with this last-named trypanosome difficult.

(¹) Laveran and Mesnil, Trypanosomes and Trypanosomiasis. Nabarro.

Table IV.—Transmission Experiments with *G. palpalis*.

Expt. No.	Duration and nature of infecting feeds.	Number of Flies.				Days fed on monkey.	Length of feeding on experiment.	Result of feeding on clean monkey.	Remarks.
		1st day.	30th day.	Dissected.	Containing flagellates.				
825	3 days on Dog 807 ...	88	73	88	2	4th-39th	Days. 41	—	No flies found with infected salivary glands.
826	3 " " 807 ...	64	53	63	3	4th-37th	42	+	Monkey infected about 34th day { 1 fly found with infected salivary glands. cf. Table II.
843	5 " Monkey 821	67	56	67	6	7th-22nd	37	+	Monkey infected about 29th day { 3 flies found with infected salivary glands. cf. Table.
844	4 " " 822	76	68	76	3	5th-18th	38	+	Salivary glands not observed. Monkey infected about 35th day { tained on dissecting the late flies of this experiment. cf. Table.
850	2 " Dog 835 ...	53	42	52	3	4th-19th	42	+	Monkey infected about 37th day { 2 flies found with infected salivary glands. cf. Table.
851	2 " Monkey 821	86	73	86	2	4th-19th	42	+	" " " 33rd day { 1 fly found with infected salivary glands. cf. Table.
852	4 " " 822	51	39	51	5	5th-17th	41	+	Monkey infected about 34th day { " " " of experiment. cf. Table.
855	3 " Dog 835 ...	66	48	64	1	4th-16th	40	+	Monkey infected about 34th day { " " " of experiment. cf. Table.
856	4 " " 821 ...	41	33	41	0	5th-15th	42	—	No flies found with infected salivary glands.
858	3 " Monkey 849	65	52	65	2	8th-13th	31	+	Monkey infected about 26th day { 1 fly found with infected salivary glands. cf. Table.
859	3 " " 822	94	57	94	3	4th-13th	40	+	Monkey infected about 33rd day { " " " of experiment. cf. Table.
860	1 day on " 821	55	41	53	2	2nd-22nd	30	—	No flies found with infected salivary glands.
861	2 days on " 821	73	...	73	2	3rd-21st	27	+	Monkey infected about 25th day { 1 fly found with infected salivary glands. cf. Table.
863	2 " " 821	92	...	92	1	3rd-18th	24	—	No flies found with infected salivary glands.
864	2 " " 822	78	...	78	4	3rd-17th	25	..	cf. Table ... " " " 1 fly found with very early infection of salivary glands.
870	2 " " 821	103	...	103	2	4th-18th	20	..	No flies found with infected salivary glands
871	1 day on " 857	27	...	27	0	2nd-13th	17	..	" " "
872	2 days on " 822	71	...	71	5	4th-10th	13	..	" " "

Table V.—Description of positive flies of Table IV.

Date.	Number of Fly.	Age of Fly when dissected.	Sex.	Expt. No.	Region of Gut.					Remarks.
					Hind.	Thoracic up to Proventric.	Proventriculus.	Salivary gland.	Proboscis.	
		Day.								
17.9.12	1	4th	♂	861	+++	-	-	-	-	
23.9.12	2	7th	"	863	+++	+++	-	-	-	
6.9.12	3	7th	"	843	++	+	+	-	-	
6.9.12	4	7th	"	844	+	+	-	-	-	
14.9.12	5	10th	"	852	+++	-	-	-	-	
23.9.12	6	11th	♀	860	+++	+	-	-	-	
16.9.12	7	12th	♂	852	+++	+	-	-	-	
9.9.12	8	12th	"	843	+++	+	-	-	-	
14.9.12	9	12th	♀	850	+++	++	-	-	-	
7.9.12	10	13th	♂	872	++	+	-	-	-	
"	11	"	"	"	++	++	+++	-	-	
"	12	"	"	"	+++	+++	++	-	-	
"	13	"	"	"	++	++	-	-	-	
"	14	"	"	"	++	+	-	-	-	
3.10.12	15	"	♀	870	++	-	-	-	-	
18.9.12	16	14th	"	852	+++	-	-	-	-	
31.8.12	17	"	♂	825	+	-	-	-	-	
22.9.12	18	"	♀	859	+++	+++	+	-	-	
4.9.12	19	18th	"	825	++	+++	+	-	-	
8.10.12	20	"	♂	870	++	+++	+	-	-	
7.9.12	21	21st	♀	826	+++	+++	+++	-	-	
30.9.12	22	22nd	"	859	+++	++	-	-	-	A few flagellates seen in sucking stomach.
6.10.12	23	24th	♂	860	+++	-	-	-	-	" " "
12.10.12	24	25th	"	864	+++	+++	++	-	-	
"	25	"	♀	"	+++	+++	++	-	-	
"	26	"	"	"	++	+++	+++	+	-	
"	27	"	♂	"	+++	+++	+++	+	-	
10.10.12	28	27th	♀	861	+++	+	+++	+++	-	Monkey became infected.
4.10.12	29	30th	"	852	+++	++	-	-	-	
8.10.12	30	"	♂	858	+++	+++	+++	-	-	1 flagellate seen free in hypopharynx near bulb of proboscis. cf. <i>infra</i> .
9.10.12	31	31st	"	"	+++	+++	0	+++	-	Monkey became infected.
20.9.12	32	34th	"	826	+	++	-	-	-	
4.10.12	33	37th	"	843	+++	+++	+++	+++	-	" " "
"	34	"	"	"	+	++	++	+++	-	" " "
"	35	"	"	"	++	+++	+++	+++	-	" " "
"	36	"	♀	"	+++	+	-	-	-	
7.10.12	37	38th	"	844	+++	+++	+++	0	-	" " "
"	38	"	"	"	+++	+++	+++	0	-	" " "
15.10.12	39	40th	"	855	+++	+++	++	+++	-	" " "
18.10.12	40	"	"	859	+++	+++	+++	+++	-	Monkey became infected. This fly became infected on Guinea-pig 898.
15.10.12	41	41st	♂	852	+++	-	-	++	-	Monkey became infected.
14.10.12	42	42nd	"	850	+++	+	+	+	-	" " "
"	43	"	♀	"	+++	++	-	++	-	" " "
28.9.12	44	"	"	826	+++	+++	+++	+++	-	" " "
14.10.12	45	"	"	851	+++	+++	0	+++	-	" " "
"	46	"	♂	"	+++	+++	-	+++	-	" " "

The evidence supplied by this table is quite in keeping with that of Table IV. With one exception, every monkey which was fed upon by a fly showing flagellates in the salivary glands became infected.

Flies number 17 and 29 show that in certain cases the development of flagellates in the fly may proceed more slowly than usual. Neither of these flies had a chance of picking up parasites secondarily from the monkey. Fly number 32 also illustrates this point as the monkey upon which Experiment 826 was fed did not become infected until the 34th day of the experiment, trypanosomes being first seen in its blood 7 days later.

Fly number 36 illustrates what is almost certainly a secondary "pick up" infection from the monkey, which became infected on the 29th day of the experiment. Flies number 41, 43, and 46 show negative proventriculus and positive salivary glands, a point already recorded in connection with *T. nanum*¹; and fly number 42 shows a very early stage of the invasion of the salivary glands occurring unusually late in the cycle. The most interesting flies of the table are, however, numbers 26 and 27, which also illustrate the stage of invasion of the salivary glands from the gut. It will be seen that the proventriculus in both these flies was swarming with flagellates, while in fly number 42 this structure contained but few organisms. These irregularities in the distribution throughout the fly's alimentary tract are quite intelligible when it is remembered that the ultimate goal of the flagellates is the salivary gland. After establishing their base in the hind and foregut the flagellates advance as best they can towards the openings of the gland ducts, *viâ* the proventriculus. Whether or not they take up prolonged residence in this organ depends apparently upon the conditions with which they are confronted. In Table V. the earliest invasion of the salivary gland occurs on the 25th day when flagellates were seen in the proximal part of the gland and only rare colonies of attached individuals were to be seen further back, no forms were, however, to be found posterior to the proximal half of the organ. These two flies were apparently unable to infect their monkey. In Experiment 858 of Table IV. it will, however, be noticed that the flies became infective on the 26th day of the experiment, so that it appears probable that 26 days is the minimum time for the cycle in the fly to reach completion.

In view of the concise and powerful evidence supplied by Tables IV. and V. pointing to the salivary glands as the seat of the infecting form of this trypanosome, it will be well to consider briefly the established facts in connection with fly transmission and to endeavour to learn their true significance.

Among the methods of diagnosing trypanosome species at present in vogue little or no attention has been paid to the question of the intermediate host, the principal tests employed being morphology, cross immunity, and the animal reactions. If, however, the various pathogenic mammalian trypanosomes at present described are in reality true species it must be borne in mind that under natural conditions the usual method of

(¹) Proc. Roy. Soc. B, vol. 85. pp. 4-9. 1912.

propagation from mammal to mammal is by the agency of biting insects. Cyclical development in glossinæ has now been proved for the great majority of the mammalian types of Africa, and direct transmission by infected feeding has been shown to be relatively unimportant. It is indeed no exaggeration to say that failing the insect intermediary or man's interference with the syringe the great majority of mammalian trypanosomes would be doomed to destruction, unless they succeeded in elaborating some new means of propagation at present unknown.

In the ordinary course of events a new trypanosome, isolated perhaps in Central Africa as the cause of some disease in man or his animals, is despatched as soon as possible to a European laboratory. Here the tests referred to above are applied and comparisons instituted with other stock strains, and finally some diagnosis is pronounced. The trypanosome strain is kept up by subinoculation in a series of animals and becomes in turn a standard for future reference. In this way a strain may be kept up for 9 years¹ without having had access to the natural transmitter, the appropriate biting insect. With such notoriously unstable organisms this method is bound to prove unsatisfactory, and the present state of the question of trypanosome diagnosis bears this out. Even in those laboratories where experiments in fly-transmission are carried out the results obtained have, so far as I am aware, rarely been applied to test the identity of two similar strains. That such a test is of considerable value I hope to establish by a consideration of the available evidence, even if purely biological considerations have not hitherto suggested its trial.

The recent results published by Roubaud and his fellow-workers, Bruce, Rodhain, Taute, &c., and also those obtained at this laboratory, prove conclusively that the different species of mammalian trypanosomes do not all take up the same position in the alimentary tract of the intermediate host. Personal experience enables me to speak of 6 different species, viz., *T. gambiense*, *nanum*, *vivar*, *pecorum*, *uniforme*, and the subject of this paper. From a study of the development of all these types in *G. palpalis* it is obvious that each species takes up what may be termed its "anterior station" before completing its development in the fly, which for the species named is either the proboscis or the salivary glands. In no case have developing flagellates been found in both situations simultaneously, although the conclusion has been severely tested both by Miss Robertson and by myself by most careful dissections. On very rare occasions one or two trypanosomes have been seen free in the proboscis lumen (hypopharynx) in flies showing a salivary infection, but these almost certainly came either from the gut or the glands. In this connection may be quoted the trypanosomes which can be found in the drop of fluid obtained by irritating the proboscis of a fly infected with *T. gambiense*, although this species is definitely known to have no development in the proboscis of *G. palpalis*. Of the above mentioned species *T. uniforme* and *T. vivar* are exclusively proboscis forms;

(¹) Laveran and Nattan-Larrier. *cf.* S.S. Bullet, No. 34. p. 53.

T. pecorum and *T. nanum* show "infection totale" *sensu* Robaud, and *T. gambiense* and the subject of this paper, develop in the gut and salivary glands.

On glancing at the results of other workers on the subject of fly transmission a difficulty at once arises in that the great majority of such experiments contain no reference whatever to the condition of the salivary glands. These are apparently overlooked in dissection. Thus, of the workers above referred to, Bruce and his collaborators and Taute are the only ones who mention the finding of flagellates in the salivary glands. As far as they go all these experiments are in strict keeping with the ideas put forward above. Thus Roubaud and Bouet¹ find *T. cazalboui* carried by *G. palpalis*, *tachinoides*, *morsitans*, and *longipalpis*, in all of which flagellates are only present in the proboscis. The same workers² transmit *T. pecaui* by *G. tachinoides*, and *longipalpis* and *morsitans*, and in all positive flies dissected flagellates extend throughout the gut tube and proboscis, *i.e.*, "infection totale."

Rodhain and his collaborators³ find *T. cazalboui* transmitted by *G. morsitans*, and flagellates are present only in the proboscis; while *T. congolense* carried by *G. morsitans* shows "infection totale" of Roubaud. Taute⁴ transmits *T. gambiense* by *G. morsitans* and describes swarming salivary glands.

Comparing the various species of trypanosome dealt with by these observers with those of the Mpumu laboratory, we have:—

- (1) "Infection totale" of proboscis and gut,

T. congolense.

T. pecorum.

T. nanum.

T. pecaui.

- (2) Proboscis infection only,

T. vivax.

T. uniforme.

T. cazalboui.

- (3) Infection of gut and salivary glands, none in the proboscis,

T. gambiense.

T. rhodesiense.

This trypanosome.

Thus trypanosomes generally admitted to be very nearly allied, as *T. pecorum* and *T. congolense*, behave similarly in the fly, as also do *T. vivax* and *T. cazalboui*.

Another striking point which is evident from the above is that the type of development in the fly for a given species of trypanosome is apparently constant, irrespective of the species of tsetse concerned. Thus *T. cazalboui* frequents the proboscis only of *G. morsitans*, *tachinoides*, *palpalis*, and *longipalpis*; *T. gambiense* develops in the salivary glands of *G. morsitans* and *G. palpalis*; the *congolense-pecorum-nanum* type of trypanosome

(¹) S.S. Bullet., Nos. 21 and 31.

(²) S.S. Bullet., Nos. 21, 22, and 31.

(³) S.S. Bullet., No. 35.

(⁴) S.S. Bullet., No. 31.

occupies both gut and proboscis in *G. morsitans* and *G. palpalis*; and *T. pecaudi* of Roubaud shows "infection totale" in *G. palpalis*, *tachinoides*, and *palpalis*, although some doubt has been thrown upon the occurrence of development in the last named glossina. (Cf. *Sleeping Sickness Bulletin* No. 22.)

All these facts tend to show that the distribution of the mammalian trypanosomes in their glossina host affords valuable evidence in considering the question of diagnosis. At the present time a glance at the literature will reveal the fact that what to one observer are separate species, to another are one and the same organism. This is evident in the case of *T. vivax* and *T. cazalboui*; *T. brucei*, *T. "dimorphon,"* and *T. pecaudi*; *T. congolense* and *T. pecorum*.

In all these cases the final court of appeal seems at present to be Laveran's cross-inoculation test, though Bevan¹ points out mere variations of strain may in this way conceivably earn the distinction of separate species. The fact that in a number of instances the so-called immune animals have been immunized against a strain which has already been vitiated by years of upkeep by subinoculation would seem to deprive it of much of its importance. Why should such laboratory strains be considered representative of a species which in nature would, at each change of host, undergo the controlling influences of the development in an insect?

Until some means is devised to place the subject of trypanosome diagnosis on a more satisfactory footing, I would suggest that more attention be concentrated on the behaviour of a given organism in the insect host. It may thus prove possible at the very outset of an investigation to separate two species which, from a laboratory point of view, show puzzling resemblances. At all events the test is a natural one which must play an all important part in the propagation of the various species of mammalian trypanosomes under natural conditions.

To apply the above principles to the present case. The evidence so far considered points towards *T. pecaudi* as the diagnosis, but the facts available on the transmission of this species are not in keeping with this opinion. Thus Roubaud and his fellow-workers have transmitted *T. pecaudi* by *G. longipalpis* and *G. tachinoides*, and with one doubtful exception have failed with *G. palpalis*. This failure may, of course, be due to local conditions, as Bouffard² considers *G. palpalis* to be the chief carrier of *T. pecaudi* in nature. But in such cases of successful transmission where the flies were dissected, trypanosomes were found throughout the alimentary tract and proboscis. No mention is made of the salivary glands, but in view of the Mpumu experience referred to above there is no reason to doubt that they were negative. Now the trypanosome of this paper does not develop in the proboscis, but has its "anterior station" in the salivary glands. Only on one occasion was a single trypanosome found in the hypopharynx, and this at a date when the invasion of the glands from the gut was expected. If the above theory as to the value of the fly test is correct, it is impossible

(¹) S.S. Bullet., No. 38.

(²) Ann. de l'Inst. Past. Jan., 1908.

to suppose that the trypanosome of this paper is identical with the species described by Roubaud as *T. pecauidi*. It is not possible to make any comparison with the development of *T. brucei* in *G. palpalis*, as Fischer¹ unfortunately makes no reference to either the proboscis or the salivary glands in his infected flies. The lack of necessary information concerning the other trypanosome mentioned in this diagnosis prevents any definite conclusion being drawn from this section. It will be well now to consider briefly the various points emphasized throughout the preceding sections and to endeavour to arrive at an opinion as to the nature of this trypanosome.

The evidence of morphology suggests *T. rhodesiense* or *T. pecauidi* and possibly *T. equiperdum* (*Sleeping Sickness Bulletin* 40), though in this last apparently no forms were seen showing the crithridial arrangement of the nuclei. As Bruce points out (*Sleeping Sickness Bulletin* 39) the posterior nuclear type of trypanosome could hardly have escaped notice if it occurred in *T. brucei*.

The animal reactions correspond roughly with those of the *T. pecauidi* of Bouffard, the occurrence of a long incubation period in guinea pigs being observed by this worker. A striking dissimilarity exists in the course of the disease in donkeys, which Bouffard describes as very chronic. On the other hand, a strain of *T. brucei* might give similar results with the exception of the guinea-pig results. *T. rhodesiense* would seem to be excluded by the chronic course of the disease in sheep and goats together with the absence of any sign of œdema.

The *T. pecauidi* of Laveran is distinguished from the present trypanosome by the guinea-pig reaction.

This trypanosome is susceptible to human serum especially if administered protectively. In this it shows a resemblance to *T. brucei* and *T. rhodesiense*.

The readiness with which *G. palpalis* can carry this trypanosome strengthens the resemblance to Bouffard's *T. pecauidi*, while distinguishing it from the *T. pecauidi* of Roubaud. It is thus possible that there exist two separate species of trypanosomes at present passing under the name of *T. pecauidi*. The guinea-pig reactions given by Laveran and Bouffard respectively for *T. pecauidi* are dissimilar in that the latter found the incubation period in these animals to be never less than 20 days, while some of Laveran's guinea-pigs died in 18 days. Again Bouffard is strongly of opinion that *G. palpalis* is the carrier of *T. pecauidi* in his district, and Roubaud is equally convinced that this tsetse plays practically no part in the transmission of his trypanosome.

Until some additional information is available as to the reasons which led to the diagnosis of *T. pecauidi* by Bouffard and Roubaud I consider that the subject of this paper should not receive a specific name. Should the above suggestion be borne out, one of the two species will have to be renamed.

It may be said in conclusion that this organism shows the general characters of the *brucei* group; it causes an acute disease

(¹) Zeit. für Hyg. u. Infectious Krank. Vol. 70. 1911.

in dogs, monkeys, rats, and donkeys, and a chronic infection in sheep and goats. A curious feature is the relative insusceptibility of guinea-pigs, only one out of 7 inoculation experiments succeeding, and this after an incubation period of 44 days.

The trypanosome is very readily carried by *G. palpalis*, in which its "anterior station" is in the salivary glands. It is susceptible to the action of human serum and shows in certain experimental animals a considerable number of posterior nuclear forms.

It appears to be similar to the organism described by Bouffard as *T. pecauidi*, though it is much more rapidly fatal to donkeys; this observer, however, unfortunately gives no details of the development of his trypanosome in *G. palpalis*.

Mpumu,

November 4th, 1912.

Note on the Life Cycle of the Trypanosome in the Alimentary Tract of Glossina palpalis.

The development of this trypanosome in *G. palpalis* is in the main very like that of *T. gambiense*, nevertheless there are certain differences that would enable an observer sufficiently well acquainted with the conditions of the latter cycle to distinguish the two forms. These differences are connected with the cycle as a whole rather than with the morphology of any one stage and will be discussed when the actual development has been described.

The earliest case of unmistakeable development was in a fly on the 3rd to 4th day of the infection. The trypanosomes had increased somewhat in size and bulk and were extraordinarily uniform in type, the membrane was a little narrower than in the blood type, the free flagellum was short and the kinetonucleus lay somewhat anterior to the posterior extremity of the body (see Figs. 1-7).

Division was in active progress, and the infection showed quite remarkably high numbers and had spread throughout the "hind" and "midgut." (These terms are used in the sense of the 1908 Uganda Commission.) The process of division is very clear and is shown in Figs. 2-7, which are from the fly here referred to, *i.e.*, they have been 3 to 4 days in the gut of the fly. The details of division are identical with those in *T. gambiense*, it will be noted that the basal granule (blepharoplast of Minchin) acts as the centrosome for the division of the kinetonucleus which precedes the division of the trophonucleus. The centrosomes for the division of the trophonucleus are of intra-nuclear origin, as in the case of *T. gambiense*. The final stage of division of the body is shown in Fig. 7, and is practically transverse exactly as in the sleeping sickness trypanosome. The equal splitting up of the body which culminates in the products lying kinetonucleus to kinetonucleus never occurs here, or for that matter in any of the four mammalian trypanosomes whose fly-cycles have been studied at Mpumu. It may be mentioned in passing that so closely do the details of division in the fly correspond in *T. nanum*, *T. pecorum*, *T. gambiense*, and the form at present under

discussion that one set of figures could be used to illustrate the process in all four cases without altering a single detail.

The forms seen in these early days are without exception fully developed trypanosomes, and the suppressed crithidial forms described during the earliest divisions in *T. gambiense*, in an earlier paper were never seen in the present case.

By the 7th day the trypanosome population in the mid and hindgut is less uniform, and lengthening forms begin to appear. Certain very characteristic types are found about this period, they are distinguished by the body being drawn out rather sharply and stiffly posteriorly and in the living state they have a curious stiff vibratile motion suggesting crithidial forms; they are, however, not crithidia but trypanosomes (see Fig. 12). From this time forward long slender trypanosomes are to be seen in the gut and later, *i.e.*, about the 14th day, they begin to appear in the proventriculus where they form the predominant and usually, but not always, the only type (Figs. 14 and 16).

At this stage in the cycle there is often a rather strikingly long period during which the development does not progress any further although no diminution in numbers has been observed. There are the usual periodic displacements of the trypanosomes from the proventriculus just as in *T. gambiense*, and also the occasional temporary overflowing into the sucking stomach of the long slender proventriculus forms. The invasion of the salivary glands occurs from the proventriculus by way of the hypopharynx, but even allowing for the wide variations in time characteristic of trypanosome cycles, it seems very considerably delayed in comparison with the *T. gambiense* conditions, more especially when the generally high numbers of the infections are taken into account.

In late flies after the 30th to 35th day occasional diminution in the numbers of parasites in the hindgut may be observed, but it is very rarely seen to occur in the midgut.

The actual presence of a slender free-swimming^o proventriculus type was observed in the proximal part of the hypopharynx of a fly on the 27th day, the salivary glands were as yet uninfected although another fly from the same box showed trypanosomes in the glands. This circumstance coupled with the fact that the early infection of the glands occurs in the ducts where slender attached forms are seen before the typical broad salivary gland types are found further back, goes to show that the glands are definitely invaded from the hypopharynx by proventriculus forms. This is also supported by the late appearance of the parasites in the glands and by the entire absence of any evidence of the trypanosomes passing through the wall of the gut.

The presence of the trypanosomes in the hypopharynx before the glands are infected, both in this trypanosome and in *T. gambiense*, is of the utmost importance, not only in that it shows the method by which the glands are invaded, but because it affords in addition a striking demonstration of the absolutely essential nature of the salivary development. It is clear that trypanosomes which are actually present in the free state in the hypopharynx have an opportunity of reaching the blood of the vertebrate host, that is to say there is no conceivable impediment

preventing the proventriculus types when once arrived in the hypopharynx from reaching the blood stream of the vertebrate. The fact, abundantly shown both here and in *T. gambiense*, that infection of the mammalian host does not occur until the glands are invaded in connection with the observation just made, seems to prove conclusively that the development in the glands is an absolutely essential part of the cycle and cannot be considered as an adventitious or unimportant manifestation.

The actual development of the trypanosomes in the glands resembles that of *T. gambiense* and is characterised by the production of well marked crithidial types. There is clear evidence that trypanosomes like that shown in Fig. 17 may be considered as the earliest stage in the gland, and there are also cogent arguments for holding the forms shown in Figs. 31-34 to be the final types, *i.e.*, the infecting individuals, but the exact sequence of forms lying between them is naturally somewhat of a matter of conjecture. I have arranged them in what I consider to be the order of development. The sequence of events appears to be as follows. The slender forms attach themselves usually at first in or near the duct and may be seen in this situation in early infections. The flagellates broaden in shape and divide and seem to migrate backwards along the gland where they re-attach themselves. They have assumed the crithidial state and may be quite rounded in shape, as for instance the specimen in Fig. 24. A good deal of degeneration goes on in the glands and half-fused agglutinating and much vacuolated specimens may be seen; a certain amount of this is pretty clearly due to the actual manipulation of the glands during investigation, as the trypanosomes appear to be in a very susceptible state at this stage of development. Vacuolation, however, appears often in quite normal looking specimens and one hesitates to consider this necessarily a pathological manifestation. Multiple division (Figs. 35 and 36) seem to occur, but the specimens have often a very pathological appearance and binary fission is the usual method of multiplication.

The whole gland in the later infections becomes simply crammed with parasites, but it is interesting to note that the progressive development from crithidia to trypanosomes and the increase in the numbers of these final trypanosomes phases (*see* Figs. 27-34) is clearly correlated with the age of the infection in the gland. Thus in one fly of the 31st day there was a numerous infection in the glands but careful search revealed very few finished trypanosomes, on the other hand, flies of the 39th, 40th, 41st, 42nd, and 44th days showed numerous trypanosomes of the type drawn in Figs. 31-34, and it is clear that the infectivity of the gland depends on these forms as a fly of the 25th day, showing a slight infection of the glands in which this form was not present did not infect the monkey.¹ It may be remarked in passing that there were two flies in the same state in this box. There is one quite characteristic feature about the cycle of this trypanosome, namely, the very high numbers present in the gland and the marked delay in the arrival of the infective period. While

(¹) Fly 26 of Table V. H.L.D.

the time taken by the gland development can only be roughly estimated, it is clear that a much longer period elapses from the invasion of the gland to the moment at which the fly first becomes infective than in *T. gambiense*. No satisfactory evidence of conjugation has presented itself at any part of the cycle.

To sum up the features in which this cycle shows a divergence from that of *T. gambiense*.

(1.) The form under discussion is characterised by a rapid and numerous early development in the mid and hindgut, the early infections showing far greater numbers of parasites than in *T. gambiense*. The type of trypanosome present at this period shows a striking uniformity in shape and size.

(2.) There is in the trypanosome here described a marked pause between the first appearance of the trypanosomes in the proventriculus and their invasion of the salivary glands, this may amount to as much as 15 to 20 days.

(3.) The gland infections show far higher numbers than in the cycle of *T. gambiense*, but the development in the glands in the strain under observation occupied a considerably longer period than is the case with the sleeping sickness organism.

MURIEL ROBERTSON.

11.—SOME ATTEMPTS TO TRANSMIT *TRYPANOSOMA GAMBIENSE* BY WILD STOMOXYS; WITH A NOTE ON THE INTESTINAL FAUNA OF THESE FLIES.

H. LYNDBURST DUKE, B.A., M.B. (Camb.), D.T.M. & H. (Camb.).

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The following experiments were carried out with a view to ascertaining, if possible, whether *T. gambiense* can undergo cyclical development in stomoxys. The flies used for these experiments were wild stomoxys (*nigra* and *calciatrans*) caught on the Mpumu cattle from day to day as they grazed around the foot of the hill. It should be noted that there has been no epidemic of cattle trypanosomiasis in the immediate neighbourhood of late and that the hill cattle are in excellent condition. The flies were kept under the same laboratory conditions as the ordinary *G. palpalis* experiments, with the single exception that care was taken by placing dripping rags over the muslin of the boxes to ensure an extra supply of moisture. Under these conditions, as will be seen below, the flies could be kept alive for a considerable time.

In the following table the experiments are set forth. The flies dying during the first 4 days of the experiment were discarded, all subsequent ones being dissected as they died from day to day. From analogy with the behaviour of *T. gambiense* in *G. palpalis* it was presumed that any flagellates found in the flies after the

Dead Flies found on various days of Experiment.

Expt. No.	No. of days on T. gambiense mon. key.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.	12th day.	13th day.	14th day.	15th day.	16th day.	17th day.	18th day.	19th day.	20th day.	21st day.	22nd day.	23rd day.	24th day.	25th day.	Total dis- sected.
750	6	...	...	...	...	...	...	...	14 = nil	5 = nil 2 = cresc. + 15 = nil	3 = nil.	...	...	...	2 = nil.	1 = nil.	1 = nil.	1 = nil.	...	...	...	...	3 = nil.	3 = nil.	35
751	6	...	...	...	...	...	...	...	9 = nil	...	3 = nil.	...	...	1 = nil.	...	...	3 = nil.	...	...	...	1 = nil.	...	2 = nil.	...	34
797	2	...	...	...	2 = nil.	4 = nil.	6 = nil.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	12
798	2	...	...	2 = nil	3 = nil.	3 = nil.	3 = nil.	4 = nil.	2 = nil	2 = nil	1 = nil.	...	...	...	3 = nil.	2 = nil.	...	1 = nil.	...	...	...	...	...	...	23
800	2	...	1 = nil.	...	6 = nil.	7 = nil.	...	nil. 3 = yeast +.	6 = nil	...	4 = nil.	...	...	...	7 = nil.	1 = nil.	1 = nil.	...	...	...	...	...	...	...	39
803	2	...	1 = cresc. +	2 = nil	...	7 = nil.	4 = nil.	1 = nil.	1 = nil	...	1 = nil.	3 = 10 = nil.	...	...	1 = nil.	...	...	...	2 = nil.	...	...	...	...	...	33
806	2	...	...	...	3 = nil.	...	4 = nil.	3 = nil.	4 = nil	2 = nil	...	3 = 2 = nil.	3 = 2 = nil.	3 = 1 = nil.	3 = nil.	3 = nil.	...	...	...	2 = nil 1 { Beds + Oresc. +	...	...	...	...	31
814	2	...	...	...	...	1 = nil.	...	5 = nil.	7 = nil	6 = nil	...	...	...	1 = nil.	2 = nil.	3 = nil.	...	1 = nil.	2 = nil.	...	...	...	...	...	28
*817	2	...	...	...	...	4 = nil.	...	...	6 = nil	2 = nil	7 = nil.	...	4 = nil.	...	...	4 = nil.	...	...	...	...	...	...	...	...	27
*818	2	...	...	...	...	3 = nil.	...	...	...	7 = nil	2 = nil.	...	...	4 = nil.	...	8 = nil.	...	...	1 = nil.	1 = nil.	3 = nil.	...	...	...	29
*820	2	...	...	1 = nil	1 = nil.	...	...	2 = nil.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4
*822	2	...	...	1 = cresc. + 3 = nil.	3 = nil.	2 = nil.	1 = nil.	...	3 = nil	1 = nil	1 = nil.	1 = nil.	1 = nil.	...	...	1 = nil.	...	...	1 = nil.	...	...	...	...	...	19
*823	2	...	...	...	...	1 = nil.	...	4 = nil.	1 = nil	1 = nil	1 = nil.	...	...	...	...	1 = nil.	2 = nil.	...	...	1 = nil	...	...	...	...	14
*824	2	...	...	2 = nil	...	2 = nil.	...	...	...	...	1 = nil.	...	...	1 = 5 = nil.	...	...	...	...	...	...	...	...	...	...	11
*840	2	...	...	...	...	...	...	8 = nil.	...	...	3 = nil.	2 = nil.	4 = nil.	2 = nil.	4 = nil.	4 = nil.	1 = nil.	...	...	...	...	...	...	...	24
Totals ...	...	...	2	11	18	31	18	30	56	43	28	9	21	12	21	29	8	3	6	5	4	...	5	3	363

first 4 days would be true developing forms and not mere passive survivors from the monkey blood imbibed. As it so happened no flagellates in any way suggesting developmental forms of trypanosomes were seen in the flies dissected the experiments have therefore a definite negative value. In the event of a positive result it would have been necessary to attempt experiments with clean laboratory-bred stomoxys in order to exclude the fallacy of the flies being infected before the experiment began.

It should be borne in mind in estimating the value of these experiments as negative evidence that Miss Robertson has shown that the developmental cycle of *T. gambiense* in *G. palpalis* shows no period when trypanosomes are absent from the alimentary canal. In a fly destined to become infective trypanosomes develop continuously from the time of ingestion. It is therefore highly improbable that a fly with developmental stages of *T. gambiense* in its gut should have escaped notice. The procedure was as follows:—The wild flies were fed first for varying numbers of days on an infected monkey and then placed daily on clean monkeys until termination of the experiment at the death of the last remaining fly. None of the monkeys became infected.

In Experiment 750 to 814 of the table the flies were fed on clean monkeys the day after the last infected feed. In experiments marked * a day's starvation intervened between the last infected feed and the first feed on clean monkey. This was done out of consideration for Patton's observation that bugs fed directly after the infecting feed with Kala Azar blood never became infected.

The first group of experiments also include transmission by direct transmission. Although no flagellates having crithidial or trypanosome structure were seen in the flies dissected two interesting organisms were discovered. One a flagellate showing the structural characters of the Bodos (Fig. 1) the other a crescent-shaped body depicted in Fig. 2.



FIG. 1.

FIG. 2.



Text Fig.—Development of trypanosomes in gut of fly.

Fig. 1.—Form from gut 3rd to 4th day.

Figs. 2-7.—Division in gut 3rd to 4th day.

Figs. 8-10.—From gut 7th day.

Fig. 11.—From gut 10th day.

Fig. 12.—Form with drawn out posterior end 10th day.

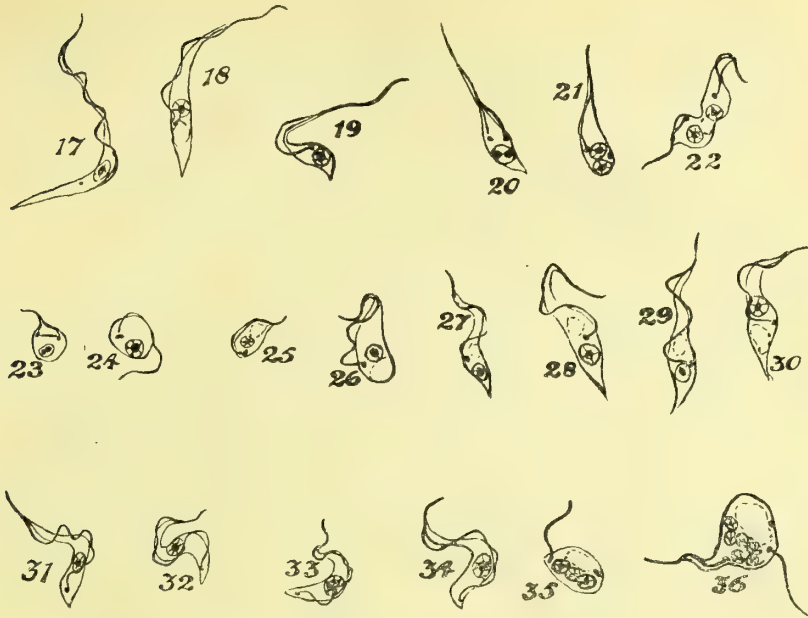
Fig. 13.—From gut 14th day.

Fig. 14.—From proventriculus 14th day.

Fig. 15.—From gut 30th day.

Fig. 16.—From proventriculus 33rd day.

Magnification = 2,000 diameters.



Text fig.—Salivary gland types.

Fig. 17.—Trypanosome newly arrived in gland 27th day.

Fig. 18.—Early crithidial form 31st day.

Fig. 19.—Crithidial type.

Figs. 20–22.—Division of crithidial types.

Figs. 23–25.—Rounded specimens.

Fig. 26.—Broad form 31st day.

Figs. 17–29.—Crithidial types apparently about to develop into trypanosomes.

Fig. 30.—Early trypanosome phase.

Figs. 31–34.—Final trypanosome stages.

Figs. 35–36.—Multiple division.

Magnification = 2,070 diameters.

It is not necessary to do more than call attention to the figures in dealing with the morphology of these two organisms. The occurrence of a Bodo-like flagellate in a blood-sucking insect is of considerable interest and, as far as I know, has never been reported before. As to the nature of the crescent-shaped bodies it is impossible to do more than theorise. They have apparently no motility whatever and are often in great numbers in the fly's-gut. It is possible that they may represent some stage of development of some organism of the sarcosporidian group, in which case their occurrence in a blood-sucking insect may be of great significance in considering the transmission of these parasites. Enquiry at the Veterinary Department in Kampala failed to elicit any information as to the existence of sarcosporidia in the domestic animals of the district. For the present therefore the matter must be left in abeyance. I hope later on to carry out more experiments on this point and also to examine *Stomoxys* from other districts.

Mpumu,

November 10, 1912.

12.—NOTES ON THE POLYMORPHISM OF *TRYPANOSOMA GAMBIENSE* IN THE BLOOD AND ITS RELATION TO THE EXOGENOUS CYCLE IN *GLOSSINA PALPALIS*.*

By Miss MURIEL ROBERTSON.

(Communicated by the Tropical Diseases Committee of the Royal Society. Received July 5, 1912.)

I. *Introduction.*

The following paper deals with the well-known phenomena of the fluctuation in the numbers of trypanosomes present in the blood of an animal infected with *T. gambiense*, and with the equally familiar question of the polymorphism of the parasites.

The relation of these factors to the production of infected *G. palpalis* is also discussed, and evidence is brought forward to show that a certain type of trypanosome is responsible for the carrying on of the cycle in the transmitting host. The actual details of the structure of the trypanosomes and the sequence of developmental stages in the fly are not touched upon in this account. They will, I hope, form the subject of a subsequent paper.

II. *General Condition of a T. gambiense Infection.*

It is advisable to consider first the fluctuation in the numbers of the parasites. It is important to note here that a close study of any given infection brings out very clearly that the multiplication of the trypanosomes occurs in the circulating blood-stream. Search has also been made for any type of multiplication in the cells of the lung, liver, and spleen, so far entirely without success. Moreover, the invariable correlation between a rise in the number of trypanosomes and a rise in the percentage of dividing forms renders such a development very improbable.

The factors controlling the numbers of flagellates present in the blood at any given time fall naturally into two categories:—

(1) Those conditioned by the vertebrate host.

(2) Those conditioned by the parasite.

Phagocytosis, alterations in temperature and the liberation in the serum of protective substances (possibly also any alteration in the viscosity of the blood) constitute the more important reactions on the part of the host likely to influence the condition of the trypanosomes, to act as inhibiting circumstances to the multiplication and to cause reduction in the number of the parasite.

The important factors in regard to the trypanosome itself are the self-conditioned processes within the body of the parasite and the production of toxic substances due to its metabolism.

By self-conditioned processes I mean such manifestations as the inherent capacity for division and nutrition and the general vigour and adaptability of the parasitic organism. The actual

* Reprinted (in part) from Proceedings of the Royal Society, Ser. B, vol. 85, p. 527.

reduction in the numbers of the trypanosomes sets in as soon as the nugatory conditions, whatever their origin, get the upper hand. It is obvious that a continual state of tension exists between the capacity of the host to destroy the parasite and the capacity of the parasite to maintain itself. If these facts are borne in mind the nature and the details of the fluctuations are more easily comprehended, though it may not be clear exactly which set of factors is at work.

The two sets of factors here mentioned form, it is clear, merely two general groups of conditions; each group represents obviously a very complex aggregate of interacting circumstances concerning which we have at present practically no knowledge.

Thus, so far from knowing what inhibits the multiplication of an organism, we have still, in spite of the very valuable work of Hertwig, Loeb, and Klebs, very little knowledge as to what are the conditions that determine the normal division of any cell. This last must be touched upon later. It is not therefore, to be supposed that the unravelling of the conditions obtaining in an infected monkey does more than afford a certain clearness and precision to our view of the picture presented.

The drop in the number of trypanosomes is often sudden, but the completeness of the clearance varies within wide limits, and the duration of the depressed period is quite inconstant.

The complex factors at work producing such a reduction may supervene at any period as regards the absolute number of trypanosomes present, but the first indication that these factors have begun to come into operation is most often a reduction in the percentage of dividing forms. The actual disappearance of the trypanosomes is, moreover, generally preceded by a shorter or longer period, during which the numbers are relatively stable and the divisions few. There are, however, occasional exceptions to this rule, and the numbers may suddenly be checked and reduced in the middle of a rise.

The mechanism by which the trypanosomes are destroyed has not been studied, but the work of other observers all points at present, as far as I am aware, to phagocytosis. One must, however, consider in passing (if only to dismiss it) the possibility of the trypanosomes entering into the cells of the host, and undergoing some form of development at this period. All the evidence is against this assumption on account of the important fact that the trypanosomes reappear relatively gradually, and their appearance is always accompanied by active multiplication in the blood stream. This fact is also of interest in showing that the disappearance is a genuine destruction and not a mere withdrawal of the parasite from the peripheral blood.

Lysis in the blood stream is also a possible explanation of the method of reduction in the numbers. There is a certain amount of evidence in favour of this to be drawn from a consideration of the endogenous cycle. It will be discussed in a later section.

The essentials of the fluctuation just sketched are repeated interminably with monotonous similarity, the only features liable to variation being the absolute numbers of trypanosomes involved and the time coefficient. That is to say, the drop may leave a

fair number of parasites in the blood, and the rise may occur without further reduction, or the drop may be very complete; it may be sudden or more gradual. The depressed period may be long or short. Thus also the rise may be more or less rapid; the exalted period may be longer or shorter; the whole process may take place with low absolute numbers, that is to say, the exalted period may not involve a very high number of parasites—this last is often very characteristic of the later months of an infection. A stable or relatively level period may proceed to a rise instead of to a drop, owing probably to the removal of some temporary inhibition.

The extreme variability of these two elements, namely, the duration of time of the various periods and the absolute number of trypanosomes involved, produces a quite fictitious appearance of confusion. The essentials of the process are in reality extraordinarily constant.

As a matter of practical technique these points are more easily demonstrated in the earlier periods of the infection when the absolute numbers are relatively high. I have worked exclusively with monkeys, but no doubt the process is very similar in other animals infected with *T. gambiense*. Superficially, guinea-pigs and rats seem to behave as monkeys in regard to the fluctuations, but no detailed study has been made of them.

The coming and going of the trypanosomes described above may be considered to be the endogenous cycle in the blood of the vertebrate.

To consider now the polymorphism of the trypanosome and its relation (1) to the endogenous cycle, (2) to the production of infected flies, *i.e.* to the exogenous cycle in the fly.

III. Method.

The method employed in studying the trypanosome is as follows. Blood-films are taken daily from a *gambiense* monkey at the same hour (9 a.m.); are fixed by exposure to osmic vapour for half a minute; are immediately plunged into absolute alcohol and left for 10 to 20 minutes. Then they are dried in air and stained with Giemsa's fluid.

This method, while it gives the worst possible picture of the nuclear detail, has nevertheless excellent qualities in regard to the type of result at present required. To begin with, it fixes all the trypanosomes present in the drop of blood, which no true "wet method" does with absolute certainty; it flattens the creatures into one plane so that they may be more easily drawn, it is rapid, the errors are apparently very uniform, and it agrees with the method employed by other workers.

Whenever possible 100 trypanosomes were drawn with the camera at 2000 magnification. They were measured by means of a compass set at a distance corresponding to one micron, as in Bruce's method. The results were tabulated, and finally plotted out on squared paper and a curve drawn. In cases where the trypanosomes were very scarce 25 or 20, and in one instance 10, trypanosomes had to suffice; it is, however, obvious that in these

cases the smaller numbers are quite as good a sample of the total forms present as 100 individuals on days when the parasites were more numerous.

The above method of measuring has been criticised by Dr. F. W. W. Stephens,* who recommends the projection of the trypanosomes on to a screen in a dark room and considers that the actual measurement should be done by the "tangent line method."

Drawing by means of the camera is obviously open to criticism, but the projection of the trypanosomes on to a screen is a counsel of perfection beyond the present achievement of workers in Central Africa. Dr Stephens' objection to the compass method is that the last fraction has often to be estimated. This last criticism is not important in the present instance, since, for example, the point 19 on the squared paper simply implies that the length of the trypanosome falls between 18.50 μ and 19.50 μ . The minute fraction of a micron need not cause undue anxiety when it is remembered to what rigorous treatment a soft protoplasmic organism has been subjected in the obviously energetic process of fixing and staining.

Moreover, it is the relative and not the absolute lengths that are significant in the present connection, and, further, in no case do the important considerations turn on very slight differences of size. All possible care has been taken with the drawings, and it is hoped that the inevitable coefficient of human error will be compensated for and evened out by the relatively large numbers of individuals involved.

By comparing the daily curves the distribution of the different types of trypanosomes in relation to the endogenous cycle can be observed with considerable precision. Cages of newly hatched laboratory-bred flies were fed daily when possible upon the selected monkey, and the nature of the trypanosome infection at the time of feeding was further studied in relation to the number of infected flies produced. Each cage was fed only once on the infected monkey, and every possible care was taken to see that the flies had actually fed. To obviate individual idiosyncrasies in the blood of clean monkeys used to nourish the flies during the experiment, the monkeys were pooled, and every cage was fed on each of the group in turn during the first 15 days. The nature of the food does not seem to affect the production of infected flies after the first days.

Owing to some other experiments being carried on at the same time in the laboratory, most of the cages were actually tested as regards infectivity, and produced typical infections in the test animals in every case in which *plus* flies were present.

IV. *Endogenous Cycle.*

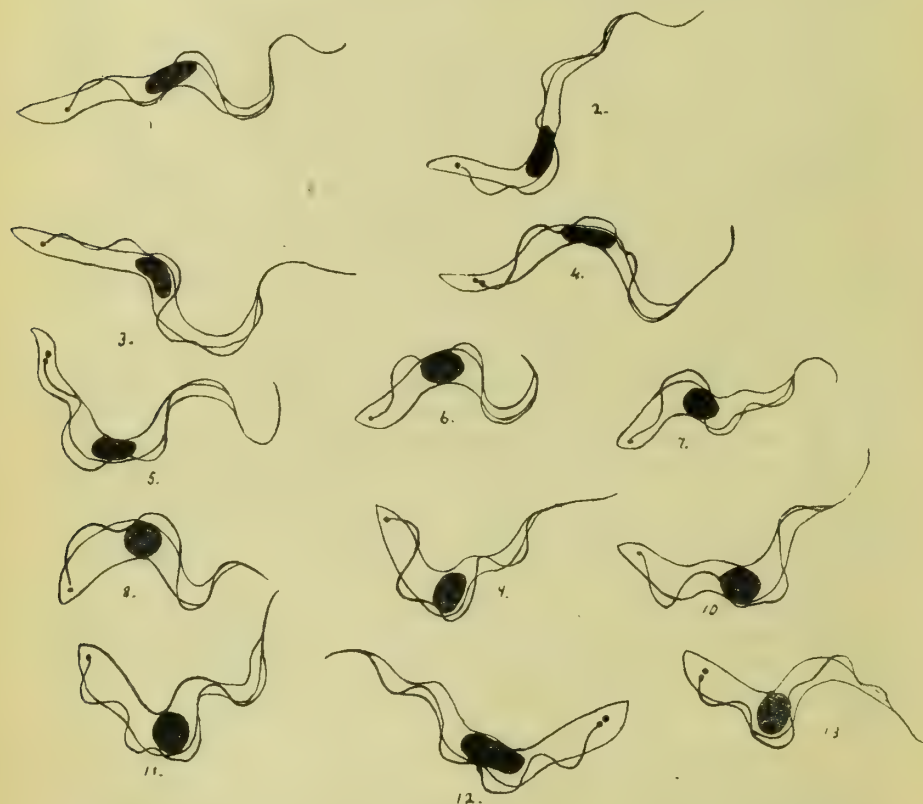
Before considering the experiments in detail, it is best to give a brief account of the general results obtained.

T. gambiense varies in length from 10 microns to 34 microns. The minimum and maximum lengths are rarely reached, the bulk

* 'Sleeping Sickness Bulletin 36,' 1912, No. 4.

of the forms varying between $15\ \mu$ and $32\ \mu$. There is really no sharp distinction into separated types, though the range of variation is relatively very wide. The trypanosomes may, nevertheless, be divided up readily enough into short, long, and intermediate individuals, but the intermediate forms insensibly join up the short and the long specimens. There is a continued transition from one type to another, but the duration of the different states varies considerably, and it is this variability of the time factor, coupled with the striking appearance of the individuals at either end of the scale, that has led to the belief that the species is really dimorphic. Such figures as 6-8 and 1-3, showing respectively short and long forms, have fostered this idea, and seem further to have suggested that the difference is an expression of sex. Neither of these hypotheses has, however, been sufficiently tested.

If one infection is carefully followed through a typical revolution of the endogenous cycle, the following conditions are found to obtain. It is convenient to choose the period immediately after a marked drop in the numbers, as the starting point. At such



Camera drawing. $\times 2000$.

FIGS. 1-3, long slender types. FIGS. 4-5, typical early division stages. FIGS. 6-8, short forms. FIGS. 9-11, intermediate forms. FIGS. 12-13, rare individuals of intermediate type in division.

a time there are very few trypanosomes present in the peripheral blood, and they are short types.* As a matter of fact, the shortest trypanosomes of the cycle are to be found at this period.

They have a short free flagellum, or may show practically no free flagellum, though it is never easy to be perfectly certain of the exact point where the body may be said to end absolutely and flagellum alone to exist. The breadth varies, but the forms are usually rather broad, measuring about 2 to 2.50 microns. In the course of the next few days there may be a level depressed period, with little or no increase in number, and there is correspondingly little change in type. As already said, the depressed period may be very short, or may last a number of days. The time factor, it must be emphasised, is open to the most capricious variation at all periods of the cycle. At the time when the first signs of a rise in numbers have occurred, it is found that with these there appears an immediate alteration in type, and long, slender, and intermediate forms are to be found, in addition to the short forms already present.

The sequence of events can be seen very clearly in cases where the depressed period shows a fair number of trypanosomes and where the rise is relatively slow; this is a quite frequent occurrence and the conditions are as follows:—

The first sign that a rise is about to take place is an increase in the size of a number of the trypanosomes; they become longer and there is a general increase in the length of the free flagellum. The width does not alter at first, and there is thus produced a state of affairs showing only short and intermediate forms, or if the increase in size is very general, intermediate forms may exist for the time being almost alone. This is an important moment in the cycle, as will be seen hereafter; some deeper physiological change in the trypanosomes accompanies the increase in size, or, rather, sets in at some period during the increase.

It is to be noted here that the rise in the numbers of intermediate forms (Figs. 9-11) at this stage occurs at the expense numerically of the short forms, and one is, it appears, in every way justified in assuming that they are derived one from the other. There now occurs a marked drawing out of the body in a proportion of the parasites, and there are thus produced long, slender individuals such as that shown in Figs. 1-3, and immediately upon this, the first burst of division occurs.

Naturally, if this process just sketched takes place very rapidly or very gradually, or with very low numbers, it is somewhat obscure, as the consecutive stages do not stand out with sufficient clearness.

As soon as the division actually takes place there is a reappearance or an increase, as the case may be, of the short forms. So that there are present long, short, and intermediate forms and dividing individuals. As the rise proceeds the relative numbers of these types vary, but there is always, as a glance at the tables

* See Tables 18 and 9. The tables referred to are not reproduced here, but are reserved for publication in a forthcoming Report of the Sleeping Sickness Commission.

will show, a relative decrease in the numbers of the long and intermediate individuals as soon as there is a lull in the number of the divisions. At the height the divisions cease or drop to a very low percentage, and correlated with this there is a reduction in the two types just mentioned. This disappearance of the long and intermediate forms is so marked that, just before the large drop in absolute numbers sets in, there is a state of affairs in which once more the shorter range of forms is almost the only one represented (*see* Table 16).

It is rather important to note that the main drop in numbers is usually preceded by a slight but quite definite numerical reduction which seems to set in after the divisions begin to slacken, so that it appears as though the inhibition of the divisions were already accompanied by a certain amount of actual destruction of the trypanosomes, though this is not yet serious in extent. This check in the divisions before the large drop in the numbers is evidence in favour of the serum exerting a harmful influence upon the parasites.

The inhibition of division can hardly be an effect of direct phagocytosis, and suggests that a certain amount of trypanolysis may occur in the circulating blood-stream. The vacuolated appearance of the trypanosomes to be noted in some cases just before or during the drop seems to lend further weight to this view.

Now one of the most striking points in the foregoing account is that the forms about to divide are the long slender ones. Figs. 4 and 5 give the typical picture of the earliest stages. The disappearance of this form when the divisions cease is also most striking in this connection, so that one is led to the conclusion that the long types are forms in preparation for division.

Briefly therefore my interpretation of the endogenous cycle is as follows:—The short forms (15-20 μ) constitute the normal adult blood type; this expression is merely used to indicate the form which has the largest duration in time in the cycle and which is the most stable. These increase in size and bulk, and form that sliding scale of individuals which may be termed the intermediate forms; these in turn lengthen out to the long, more slender types, which proceed to divide, giving rise once more to the short forms. The products of division are often unequal to a varying extent. One individual is usually much shorter, has a very short free flagellum or none at all, while the other partner may be of considerable length. Where this occurs there is a suggestion that the longer individual may proceed to divide again within a very short period, but the point is not very clear.

Early dividing individuals are found more nearly approximating to the intermediate type, but they are not numerous (Figs. 12 and 13) and appear to be simply the representatives of the lower end of the range of size at which division occurs. In very numerous swarming periods or during very rapid rise they appear to be slightly more frequent and are probably due to a tendency to telescope the consecutive stages. It is to be observed that the kinetonucleus in these types not infrequently divides at right angles to the long axis of the trypanosome instead of

parallel to it as is usual in the typical *gambiense* division. I am inclined to think that this may be an indication that the general tendency towards the drawing out in length has been suppressed in some way in these individuals. These divisions form only a very small part of the total numbers.

A sex interpretation of the above facts appears to me to be so improbable as hardly to merit discussion, and the arguments, as will be seen later, derived from the results of the glossina experiments are entirely contrary to such an hypothesis.

I am unable to suggest any reason why the trypanosomes should increase in length before division. We are at present without any clear knowledge of the internal stimuli and general factors producing division, although Hertwig's work upon the nature of the physiological tension existing between the nucleus and cytoplasm of certain organisms would possibly find an application in the present instance; but it is highly doubtful if that would afford any explanation of the alteration in the actual body form.

We know nothing as to what intensely complex factors really determine the body form of such an organism as a trypanosome. An intensive study of the physical and chemical condition of the blood at the different periods might reveal some external stimulus inducing the division of the parasites, and this might or might not afford the explanation of this particular change in shape. To maintain in the face of the facts brought to light by the study of the endogenous cycle that the long forms are males and the short forms females is obviously unreasonable. It would lead to such an absurd statement as that the males were responsible for the endogenous multiplication, the real fallacy being that we have at present no basis upon which to distribute the sex labels.

The point now arises as to whether there is any observable difference which might be attributed to sex among what I have termed the "adult types." There is a certain variation in length and breadth among these forms, but it is not marked, and there is no evidence so far as I have seen for attributing these trifling differences to sex. This leaves, of course, the question of actual conjugation quite unprejudiced for two very important reasons:—

(1) Flagellates both may and do conjugate without any external visible difference in the gametes.

(2) If differentiation of gametes does take place it may not occur until the forms are ingested by the transmitting host.

The next section, which deals with the infectivity to fly of the blood of these different phases of the endogenous cycle, affords further evidence in regard to the question of sex.

It may be pointed out in passing that a clear view of the endogenous cycle reveals a wide range of difference in the physiological state of the trypanosomes at different times, and it is clear that this is worthy of consideration from the therapeutical aspect of the trypanosome problem. The introduction of a drug would probably produce somewhat different effects, according to the moment of the cycle chosen for its application.

V.—*The Relation of the Endogenous Cycle to the Production of the Infected Fly.*

The chief point brought forward by this aspect of the question may briefly be stated as follows:—

Is there any definite condition either of the individual parasite or of the infection as a whole requisite for the production of infected glossina?

In a previous paper it has been recorded from experiments treated in a different manner,* that negative periods in relation to fly occurred, although trypanosomes might be present in the blood at the time of feeding. Moreover, these experiments pointed towards the trypanosomes rather than the fly as being responsible for the negative result. The present work has confirmed this general observation.

Failure to infect fly must depend on some one or other of the following factors:—

(1) Failure of the flies to feed; this may be called an adventitious negative, and can be obviated by keeping the flies for at least 24 hours after they have emerged, and by the exercise of patience and observation at the time of feeding.

(2) Absence of trypanosomes from the blood imbibed by the flies in the experiment.

(3) Absence of sufficient numbers of a given type (or types) of trypanosome capable of surviving in the fly.

(4) Presence of the requisite type, but in a physiological state unsuitable for survival in the fly.

(5) The capacity on the part of all the flies in the experiment of digesting all the trypanosomes imbibed.

It must here be mentioned that all the fly results are obtained in spite of a certain general tendency on the part of the flies to digest their parasites. All the experiments go to show that this negative factor may be taken as being very fairly constant. Any transmitting host whose digestion is rapid tends to show a relatively low percentage of carriers—thus mosquitoes, fleas, and tsetse flies, quite apart from the widely divergent nature of the parasites involved, all produce relatively few carriers.

Leeches and ticks, for instance, whose digestion is very slow in comparison, give, on the other hand, practically 90 to 100 per cent. of carriers.

It is advisable to take the same part of the cycle as that selected in the last section for the starting point, namely, immediately after a drop in numbers. Such a period which shows very few trypanosomes and all of the shorter type is an effective period and generally produces about the average number of *plus* flies. Now this is a result of considerable importance. There is at this time a population of trypanosomes which have just suffered a process of elimination, they have just passed through some set of conditions that has proved fatal to the vast majority. There are two things to consider: (1) the type of trypanosome which has survived, and (2) its condition. The type of trypanosome is very

* 'Roy. Soc. Proc.,' B, vol. 85, p. 241, *et seq.*

clearly the shorter individuals (*see* Tables 5, 17, 18), and they must, moreover, be those capable of resisting the particular adverse circumstances to which the majority have succumbed.

There is therefore at these periods a given type of resistant trypanosome which is, as shown by experiment, capable of infecting flies. It can therefore be concluded that not only are the short types the resistant individuals but also that this type by itself is capable of producing infection in flies.

The production of resistant strains by the use of drugs and sera has no doubt some relation to these probably temporary states of resistance occurring naturally in the untreated host. And it is not without significance to an understanding of the general biology of trypanosomes that these resistant individuals are also just those capable of carrying on the cycle in the transmitting host. This has a bearing too on the interesting discovery of the Commission of 1908, that trypanosomes persisting in antelope are rare in the blood but afford a relatively high percentage of infected flies. The low numbers and the absence of pathological symptoms on the part of the vertebrate imply that the conditions in the latter exercise a high degree of control over the parasite, and, correlated with this, the trypanosomes must in all probability be in a much more active state of resistance to their environment while sojourning in such a host. That this should also be the condition of affairs producing a large number of infected flies is of obvious importance and the connection with one another of the various instances just cited hardly needs to be further emphasised.

It has now been shown that the short forms are definitely capable of surviving in the transmitting host, but that in itself does not exclude the intermediate and long forms from a similar development. This apparently does not occur.

The days of the depressed period continue to be positive for fly until just before the rise, that is until the appearance of the intermediate forms in numbers sufficiently large to cause a serious diminution in the short forms. This period before the rise is one in which the percentage of *plus* flies produced sinks so low that none are found in the experiments—the number of flies ranges from about 45 to 100 in each experiment. In some cases this is probably an absolute negative, more often it will be only a relative negative, and if several hundred flies were fed probably an infected individual would be obtained.

The positive and negative periods in regard to the infectivity to fly shade insensibly into one another, thus there is a moment when the drawing out in length of the trypanosomes has begun which is still positive although the negative period supervenes in a short time. When divisions are actually taking place the blood is infective to fly just in proportion to the number of short forms present. A study of the tables and the analysis of the experiments given later on make this point clear. Such a multiplicative period may have a negative phase as soon as the proportion of intermediate and long forms preponderates unduly.

At the height the infectivity has a tendency to diminish, although the requisite form is present in large numbers. This

is very marked in a swarming infection showing very numerous parasites, here the swarming period is often negative—it has been a common experience to feed cages even for two consecutive days on a monkey in this state without producing a single infected fly. The reason is pretty clearly that the trypanosomes are in an exhausted state physiologically, and force is added to this when it is noted that the actual period of a drop is negative.

This last fact is also an argument in favour of the surviving trypanosomes found in the depressed period having developed their resistance during the unfavourable time. That is to say these surviving trypanosomes found immediately after a drop are not, as it were, a separate type of trypanosomes of a resistant character, but are a certain number of the ordinary adult type which have been capable of adapting themselves to the unfavourable conditions at the time of their occurrence. Were this not the case, both the swarming period and the period of the drop would be as infective to fly as the depressed period.

The above observations upon the production of the resistant trypanosomes are only what one would expect upon the assumption that the Protozoa are capable of evincing reactions corresponding in some sort to the phenomena of immunity in the Metazoa.

The very first day on which trypanosomes are to be found in the blood of a newly infected monkey falls into exactly the same category as any other active period of multiplication, and is positive or negative to fly according to whether the adult type is present in sufficient numbers or not.

While in the actual experiments here described the negative periods do not seem to be of very long duration, nevertheless in feeding many cages on a population of monkeys at random these negative states have a profound influence on the results. Thus, in a previous group of experiments, out of 62 cages fed only 29 showed infected flies.

All the above results are, it is clear, entirely opposed to a sex interpretation of the dimorphism. If the long forms were males and essential to the development in the fly then the depressed periods would not be infective. The question of sex does not seem to me to require further discussion in relation to the endogenous cycle.

VI. General Summary.

1. *T. gambiense* is a polymorphic species with a continuous range of variation.
2. The shorter forms of 14—20 μ in length constitute the normal adult blood type.
3. The intermediate individuals measuring 20—24 μ in length are growth forms and lead to the long forms of 23—33 μ , which are those about to divide. The polymorphism of the trypanosome is thus due to growth and division phenomena and does not correspond to a sex differentiation.
4. The shorter forms are those destined to carry on the cycle in the transmitting host

5. There are definite periods when the blood is not infective to fly although trypanosomes are present. Such periods are (a) just before an outburst of multiplication; (b) during the destruction of trypanosomes preceding a depressed period; (c) the summit of an exalted period involving very numerous trypanosomes—at such a time the parasites very frequently show signs of exhaustion; (d) certain periods of rapid multiplication when both the absolute and relative numbers of the shorter forms are low.

CHARTS AND ANALYSIS OF EXPERIMENTS.

The series A consists of observations of the blood of Monkey 597 for nineteen consecutive days. Monkey 597 was infected by wild flies from Kibanga. This strain was in general and over a long period found to produce both greater numbers of plus flies and more rapidly developing cycles in the fly than is usual with the Uganda strains.

The infection was tested in the customary ways, and the organism is undoubtedly the trypanosome which has always been considered to be *T. gambiense* by the Commission and workers in the Mpumu laboratory.

The monkey first showed trypanosomes on February 9, 1912. The series A was started on February 17.

The symbols used to give a rough estimate of the comparative numbers of parasites present upon the different days are as follows:—

- + indicates that trypanosomes are present in the blood, but very few in number.
- + to ++ = trypanosomes present, but not numerous.
- ++ = trypanosomes present in fair numbers.
- ++ to +++ = trypanosomes present in good numbers.
- +++ = many trypanosomes.
- ++++ = blood swarming with trypanosomes.
- = indicates that no trypanosomes were seen either on live or on stained films after a reasonable period of search. In the case of a stained film, if 30 to 45 minutes with a low power failed to reveal any trypanosomes, the film was abandoned and no drawings made.

Where not otherwise stated, each table consists of the percentage of trypanosomes of varying lengths found in the process of drawing 100 individuals. Where there is a red and a black curve, the red curve corresponds to the actual number drawn, the black to the percentages which are obviously only approximations in these cases. This has only been done, of course, in those instances in which numbers less than 100 have been drawn.

Chart I. shows few divisions—3·6 per cent.—and a curve, whose highest point coincides with the length of trypanosome equal to 19 m. Altogether the individuals, measuring between 14-20 m.

inclusive, comprise 65 per cent. of the total. The curve is remarkable in registering the shortest trypanosome found, namely, a specimen of 10 m. in length. No cage of flies was fed on this day.

Chart II. shows a typical curve of a stable period with relatively low percentages of divisions (5 per cent.) and with the highest point coinciding with the length of 19 m. 74 per cent. of the trypanosomes are between 14-20 m. in length. The cage fed on that day yielded 5 and 6 per cent. of plus flies; this is a pretty typical positive state. A fair number (+ +) of trypanosomes were present in the blood. This day, as will be seen from the following tables, is the top of this period of increase, although the absolute numbers involved are not large.

Chart III.—Here there is a diminution in the number of divisions (2 per cent.), but the most numerous trypanosomes are of 20 and 21 m. in length, and there is a drop in the percentage of plus flies (3 per cent.) produced. This curve suggests that the trypanosomes are beginning to draw out for another burst of division, which, however, does not occur, as Chart IV. appears to correspond to an actual period of reduction; there are fewer trypanosomes present, there are no divisions; the trypanosomes are in many cases vacuolated and suggestive of degeneration. A cage of 64 flies fed at this time produced no plus individuals.

Chart V. of the following day shows very few trypanosomes, only 20 being drawn, of which one is in division. The cage 68, which contained 78 flies, produced 5 per cent. of plus individuals. This curve is characteristic of the period immediately after a drop in numbers, though the depressed period is not to be of long duration, as the trypanosomes are already drawing out for a fresh rise. The highest point of the curve corresponds to a length of 19 m. The proportion of trypanosomes below 20 m. in length is large in comparison with the whole.

Chart VI. is a typical negative blood period. Here the trypanosomes are still very few in number, but they have drawn out in length, and intermediate and long forms preponderate. One individual out of the 25 drawn was in division. A cage of 46 flies yielded negative results.

The next day's Chart VII. is of interest. In the twenty-four hours between the two examinations there has been a relatively great numerical rise, so that now trypanosomes are present in good numbers (+ + to + + +); the actual divisions have, however, slackened off, and now number 4 per cent. This proves, as the next tables show, to be the highest point reached, and a short level period is being established. The forms between 14-20 m. inclusive equal only 21 per cent. of the total, but as the trypanosomes are numerous they are present in sufficiently large absolute numbers to produce a large percentage of plus flies, namely, 8.9 per cent.

Chart VIII. shows a slight drop in absolute numbers, although the actual percentage of divisions has increased from 4 per cent. to 12 per cent. The infectivity has dropped to 2 per cent., and from

the disappearance of the vast majority of trypanosomes during the following twenty-four hours one may conclude that the parasites are already in Chart VIII. struggling against the conditions which are again to check the rise. It is interesting to note that there is here a cutting down of the numbers in the middle of an obvious attempt at further multiplication.

Chart IX. marks once more the time immediately after the destruction of the trypanosomes, and once more the preponderating individuals are between 14-20 m. in length; the infectivity to fly is high—namely, 8.9 per cent. of plus flies were produced. Very few trypanosomes are present; but here again the depressed period is soon to be over, as the divisions are again setting in.

Chart X. shows the drawing out of the forms preparatory to the next rise; the relative and absolute numbers of the short forms are low; divisions number 16 per cent. of the whole, although trypanosomes are still scarce. This is, as Chart VI., a typical negative period; it is the non-infective phase of the rise. Fifty flies fed at this time produced no plus individuals.

Up to Chart IX. the infection has been fluttering backwards and forwards; there have been constant attempts at a rise always cut down by some set of contrary conditions. The absolute numbers have never been high, and the infectivity has swung between very wide limits in relatively short periods of time.

From Chart X. on there is a steady rise for some days, and the parasite definitely gets the upper hand, and the conditions are more clearly outlined.

Chart XI. shows an increase in numbers; 6 per cent. are in division. The most numerous form measures 20 m. There was no cage of flies available that day.

Chart XII. shows a fair number of trypanosomes present; an increase upon those present on the previous day; divisions equal 8 per cent. The height of the curve corresponds to trypanosomes of the length of 22 m. (*i.e.*, intermediate forms), and the shorter forms, though present, are not relatively numerous, nor are the absolute numbers yet sufficiently high to neutralise this discrepancy. Correlated with this, one finds a rather low percentage of plus flies, namely 2 per cent.

On the following day (Chart XIII.) the steady rise still continues; the trypanosomes are now present in good numbers (+ + to + + +). Divisions number 9 per cent., but the short forms greatly preponderate, and the summit of the curve corresponds to the length of 19 m. With the increase in the short forms, we find the infectivity rising once more, and 8.5 per cent. of plus flies were produced from the cage fed that day.

Chart XIV. shows many trypanosomes (+ + +) and 12 per cent. of divisions; the short forms still predominate, and the infectivity is still pretty high—5.8 per cent. of plus individuals being produced from the flies fed on that day. 19 m. is still the most numerous length of trypanosome, and the individuals of this

measurement have risen from 18 per cent. (as in Chart XIII.) to 21 per cent. of the total. In the next Chart (XV.) this length of trypanosome forms 24 per cent. of the total. The numbers have risen still further, and the parasites are now very numerous (+ + + to + + + +), divisions have fallen to 7 per cent., and there are signs of exhaustion beginning to set in. The infectivity drops to 1.3 per cent.—a cage with 75 flies producing only 1 plus individual.

Chart XVI. is of some importance. The divisions drop to 2 per cent., and with this one gets a marked closing of the curve which becomes tall and narrow; the length of 19 m. is now represented by 32 per cent. of the total. This is very significant in connection with the view expressed earlier in the paper that the multiplicative forms are the long types.

It is hardly necessary to point out that all the long types are not merely forms actually *in* the process of dividing, as a glance at the charts show that the percentage of individuals in division is markedly less than the percentage of long forms. Table XXVII. also disposes of this question.

There has been a reduction in the number of parasites in the twenty-four hours intervening between XV. and XVI., but there are still a fair number (+, +) of trypanosomes present, and this is a good example of the smaller destruction which often precedes the main clearing off of the parasites. On the following day (Chart XVII) the destruction of the trypanosomes had been so sweeping that only ten specimens could be got after prolonged searching of a 3×1 film. The chart gives the actual numbers, not the percentages. All the trypanosomes are below 20 m. in length, and it is of particular interest to note that, in spite of the very low absolute numbers present, a cage of 50 flies produced 2 plus flies, which equals a percentage of 4 per cent. The significance of such a result has already been discussed.

In Chart XVIII. the trypanosomes are still very scarce, but more numerous than on the previous day; 20 specimens were drawn, of which 1 was in division. Eighteen out of the 20 individuals measured between 13 and 20 m., and the cage produced 5.2 per cent. of flies.

The last chart of Series A (XIX.) shows the beginning of the next rise; the intermediate and long forms predominate. Divisions equal 20 per cent., and the cage fed on that day was negative. This is a quite typical negative picture, but the cage only contained 31 flies, which is not quite an adequate number.

SERIES C.

A shorter series of experiments were carried out with Monkey 642.

Monkey 642 was infected by flies from Monkey 199, which had been infected by the injection of the blood of a reedbuck. This

reedbuck had been infected with a human strain of *T. gambiense*, and had harboured it for fifteen months at the time of injection into 199. 199 produced an unusual number of plus flies, and showed virulent rapid cycles in the flies. Monkeys infected by flies carrying the 199 strain were only normally infective, producing, on the average, the 3·4 per cent. of plus flies characteristic of the usual Uganda strains. The flies, however, had infections which were markedly backward in developing and never showed very high numbers; they were, nevertheless, capable of infecting monkeys in the normal way when once the cycle was complete.

642 agreed in every way with the other strains of a similar origin. This monkey showed trypanosomes for the first time on February 15, 1912. Series C was started on March 12, 1912.

Chart XX.—Trypanosomes were very scarce. Twenty specimens drawn; 9 of these measured between 14-20 m. in length. A cage of 79 flies afforded 5 plus individuals, which is equal to 6·3 per cent. of plus flies.

Chart XXI.—Here the parasites were still rather few in number (+), though much more numerous than on the previous day. 43 per cent. of the total number measured between 13 and 20 m. The divisions equalled 16 per cent., and the plus flies amounted to 17·8 per cent. of the total. The curve is interesting as suggesting a trimorphic division of the trypanosomes; it is a transitory state; but both transient dimorphism and trimorphism are to be seen at times. The conditions already discussed seem to afford ample explanation of these phenomena.

Chart XXII. is very like XXI., and requires no further comment. The great spread of the curve may be noted in correlation with the high percentage of division, namely, 26 per cent.

Chart XXIII.—The numbers are still rising (++ to +++), and the curve is still very much spread. Infectivity is high, as is also characteristic of the two preceding charts. Divisions 2 to 4 per cent.

Chart XXIV. shows a sudden drop in number of divisions, which now equal only 4 per cent., and there is a slight diminution in numbers (++). The curve closes with corresponding suddenness, and the summit coincides with the length of 19 m. Trypanosomes of this measurement equal 26 per cent. of the total.

The absolute numbers of the parasite have never been very high, and there is no appearance of exhaustion, and in correlation with this the infectivity remains high, 8·6 per cent. of flies being produced.

By the following day there was a complete and sudden clearance, so marked that ordinary searching revealed no trypanosomes. The cage fed was negative, but was not a test, as, unfortunately, some ants broke into the cage and ate a large number of the flies.

Some further experiments were carried out, but as the results were in no way divergent they are not quoted.

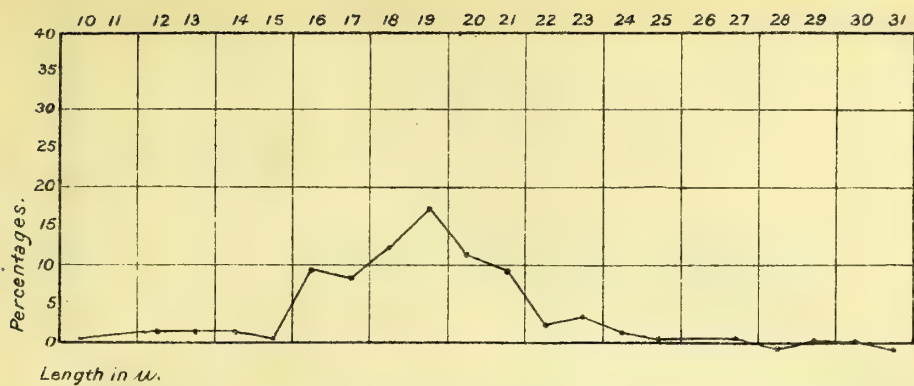
Two rather interesting charts are, however, picked out; both are from the first day on which trypanosomes were observed in the blood of two monkeys newly infected by fly.

Chart XXV.—Here the trypanosomes are not numerous (+ to ++); divisions amount to 15 per cent.; the curve is wide, and the intermediate and long forms preponderate greatly. The blood is not infective to fly; 95 flies were fed, but no plus individuals resulted.

Chart XXVI.—The trypanosomes in this case are numerous (++++ to +++++); division figures amount to 30 per cent., and the curve is very wide. The percentage of short forms is curiously enough exactly the same as in XXV., namely, 17 per cent. of the whole, but the absolute numbers are here much higher, and correlated with this one finds that the blood is infective to fly, producing 4 plus individuals out of 129 flies, which is equal to 3.1 per cent.

Chart XXVII. gives the percentages of the trypanosomes of various lengths derived from drawing 2,155 trypanosomes (none of which were in division). These specimens are all the non-dividing individuals from the various series of experiments, and represent a population of trypanosomes derived from five monkeys in groups taken upon different days. The chart shows a steep curve, and supports the view that the species is polymorphic with a continuous range of variation not truly dimorphic. Sir David Bruce, in a recent paper (Proc. Roy. Soc., B. vol. 84, 1911), gives the measurement curves for *T. gambiense* and *T. brucei*; he describes the former (with which I am alone concerned) as markedly dimorphic—a statement which the curve drawn hardly bears out. I am in substantial agreement with Sir David Bruce as to the general measurements and the types figured, but do not consider that there is any evidence of marked dimorphism. The difference in contour between the curve in Chart XXVII. and that of Sir David Bruce's paper is easily explained by the much more varied population from which I have drawn my specimens. It is not stated in Sir David Bruce's paper, but probably the curve there depicted is derived from one or two slides.

I should like to point out that the results here recorded offer an obvious criticism of the biometric method of identifying trypanosomes. A slide taken at random is clearly of no value, as a glance at the very different curves shown in the charts will at once reveal. Moreover, the number of days after infection is not a sufficiently good control of the conditions; the percentage of dividing forms would afford some basis of comparison, but even that is not entirely above reproach.

Length in μ .

Monkey 597.

III Tr drawn. N° of div. = 4 = 3.6%

No cage fed.

17/2/12.

II.

Length in μ .

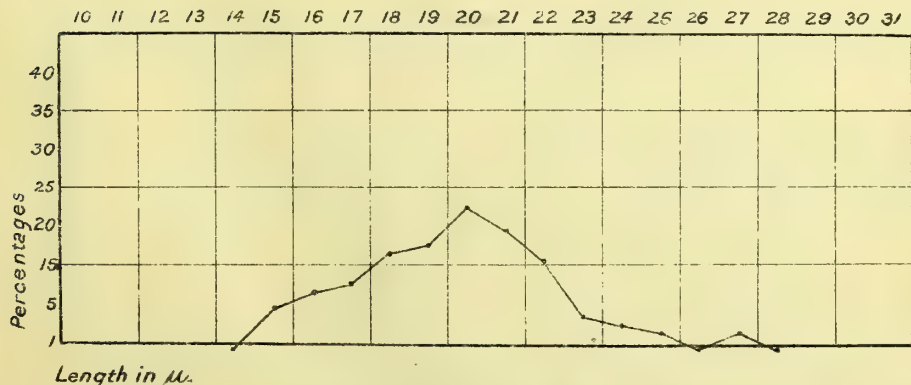
597.

100 Trypanosomes fair N° ++. div. 5%

18/2/12.

Cage 95, 143 flies 8+ = 5.6%.

III.

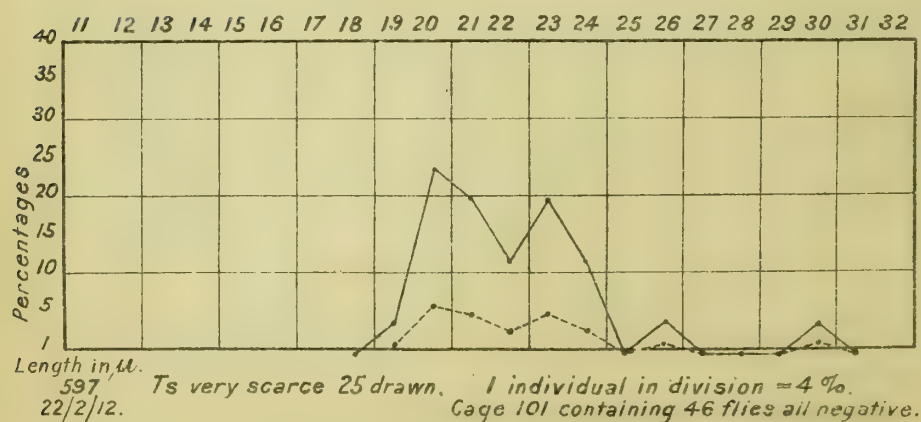
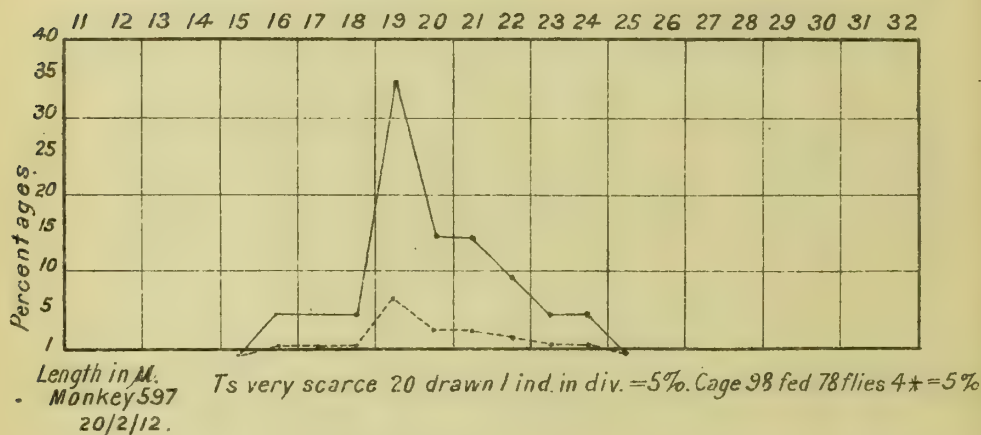
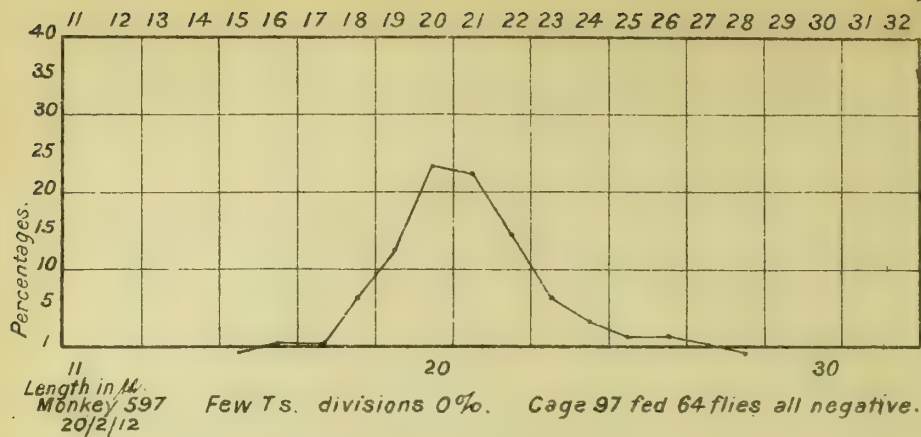
Length in μ .

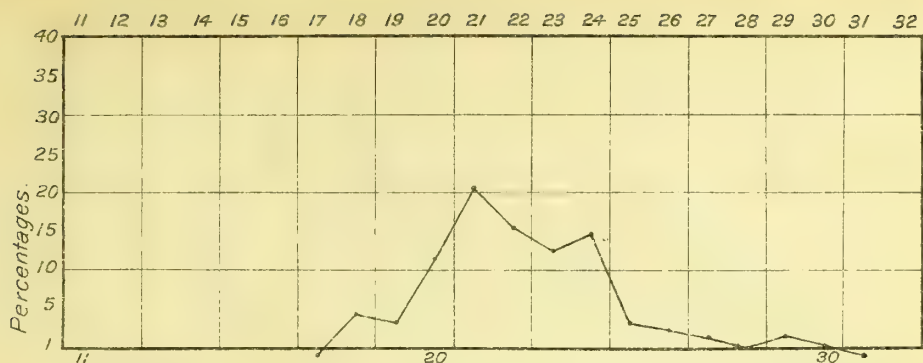
Monkey 597

2% divisions cage 96, 65 flies fed 2+ = 3% of + flies.

19/2/12.

Monkey ++.





Length in μ .

Monkey 597.

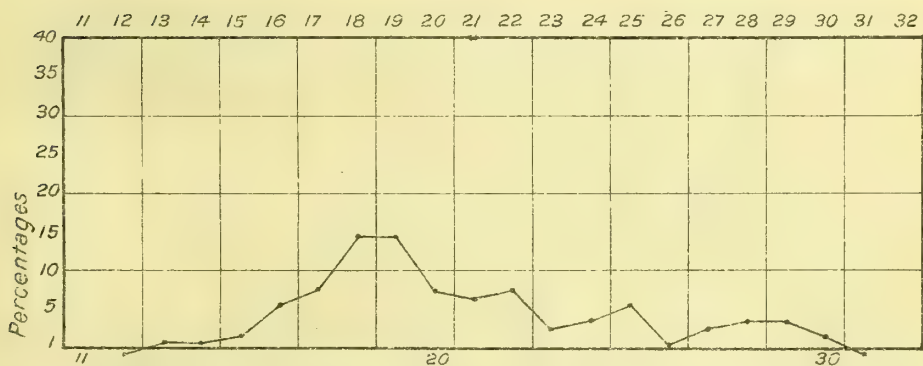
23/2/12.

division 4%.

good N° of Ts. present ++ to +++.

Cage 103 fed 56 flies, 5+8.9% +

VIII.



Length in μ .

Monkey 597.

24/2/12.

divisions 12%

Fair N° of Ts. ++

Cage 106, 45 flies 1+=2.2% of + flies.

IX.

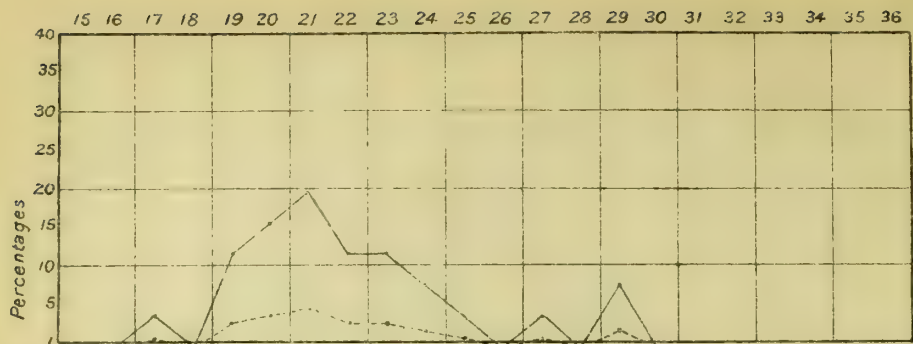


Length in μ .

Monkey 597.

25/2/12.

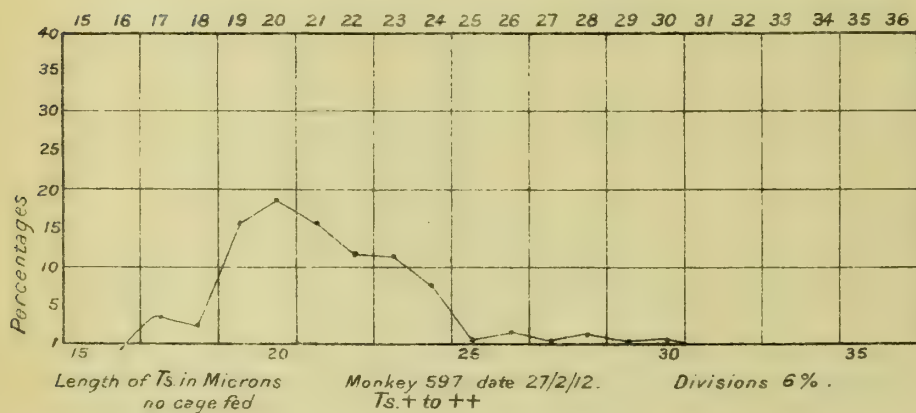
Ts. very scarce 25 drawn 2 div.=8%. Cage 107 fed, 78 flies Ts +=8.9% of + flies



Length in μ .

597 *Ts.* very scarce 25 drawn 4 in div. = 16%. (110) cage of 50 flies fed all neg.
26/2/12.

XI.

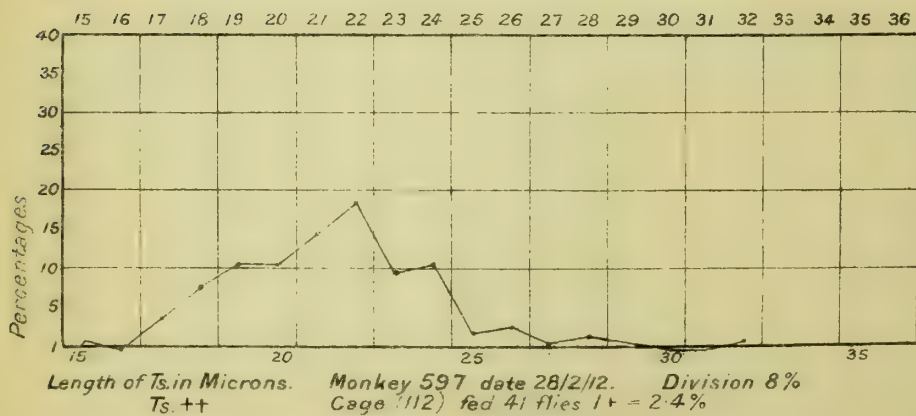


Length of *Ts.* in Microns
no cage fed

Monkey 597 date 27/2/12.
Ts. + to ++

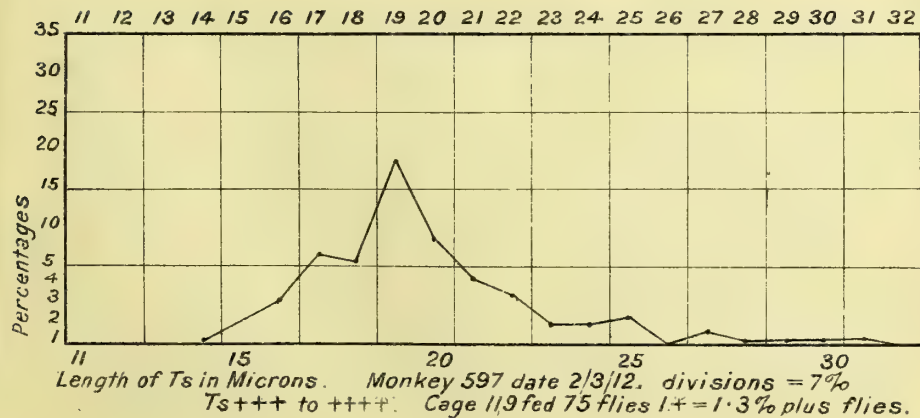
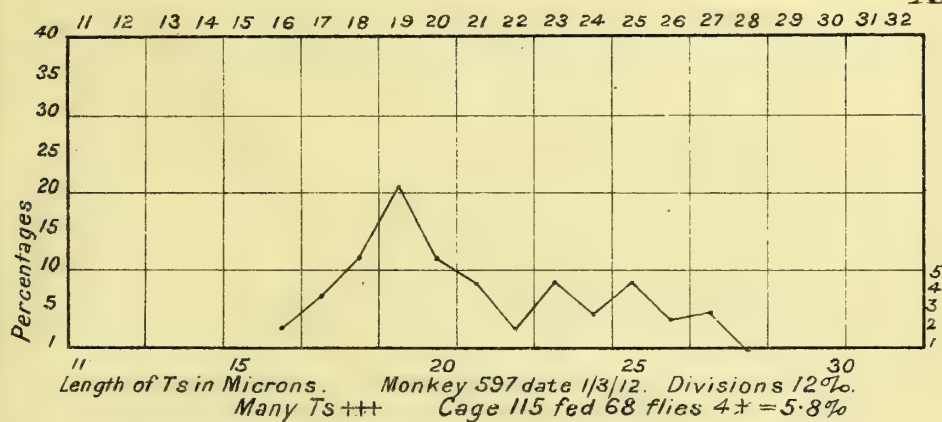
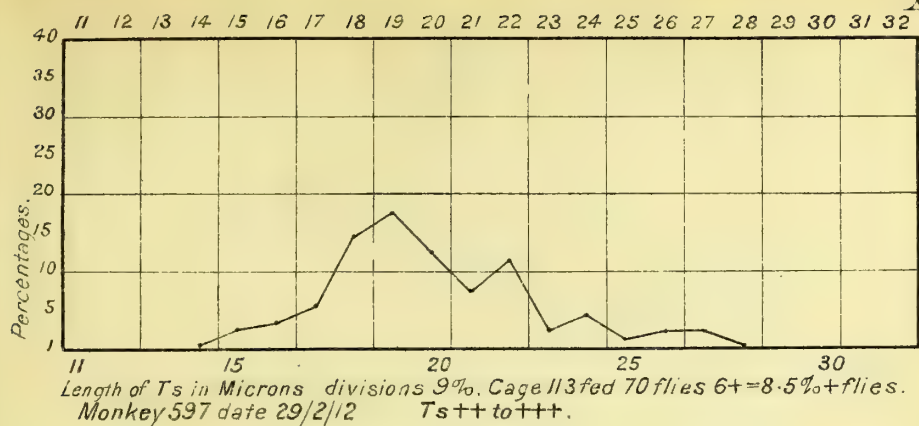
Divisions 6%.

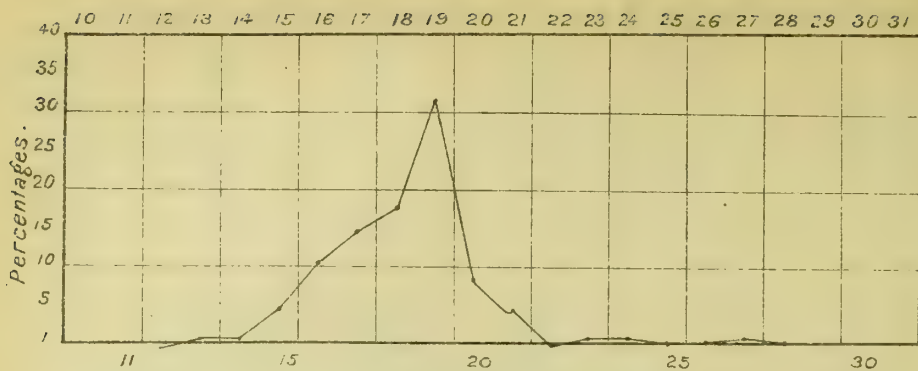
XII.



Length of *Ts.* in Microns.
Ts. ++

Monkey 597 date 28/2/12. Division 8%
Cage (112) fed 41 flies 1+ = 2.4%





Length of Ts in Microns.

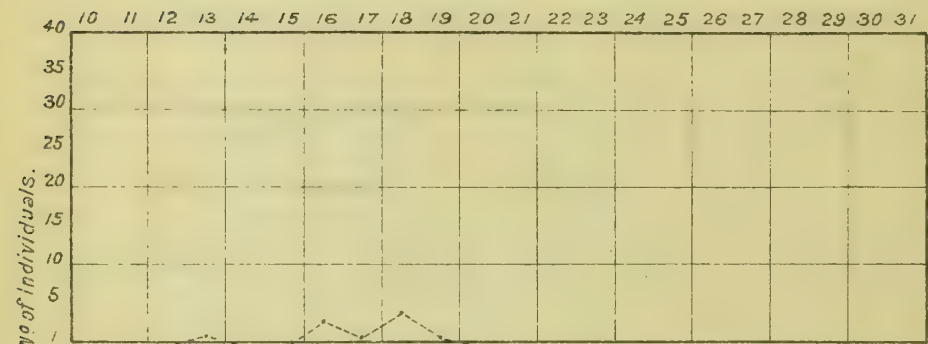
Monkey 597 date 3/3/12.

divisions 2%.

Ts $\neq$ much fewer than on 2nd

Cage 120 fed 72 flies 2 $\neq$ = 2.7%.

XVII

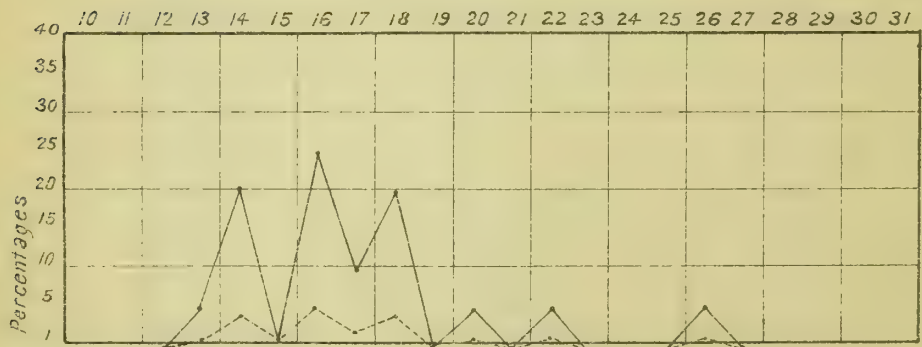


Length in μ .

597. Very few Ts indeed, 10 got with great difficulty. Cage (121) fed 51 flies 2 $\neq$ = 4% of $\neq$ flies.

4/3/12.

XVIII

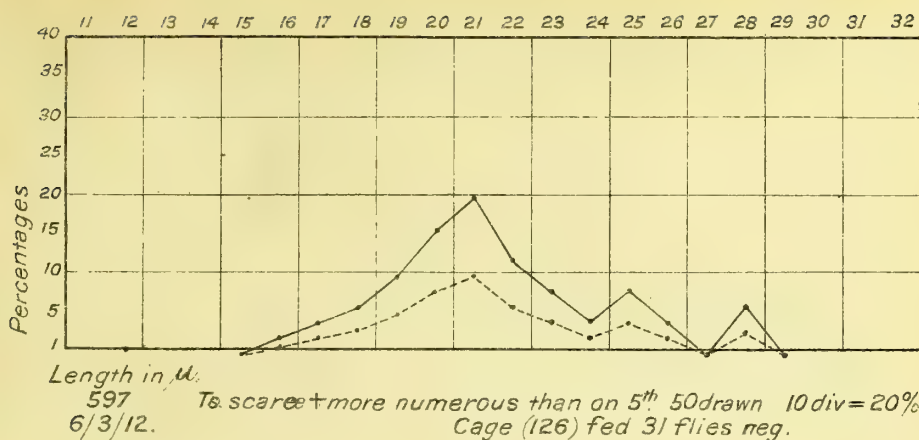


Length in μ .

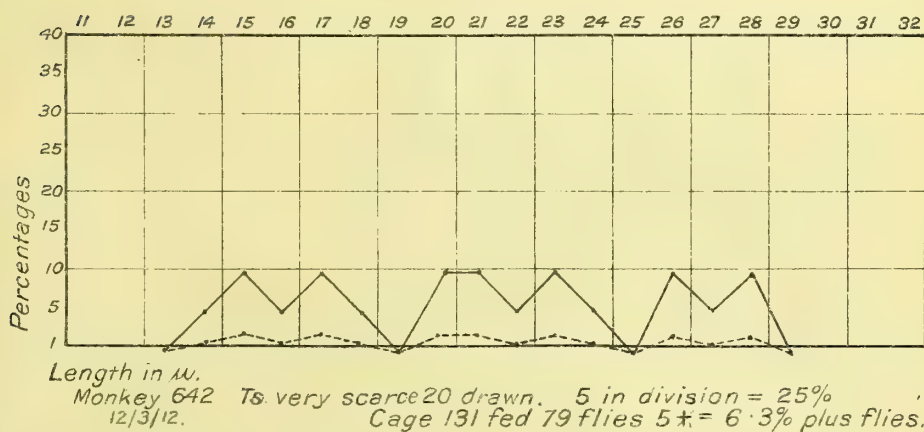
Monkey 597

5/3/12

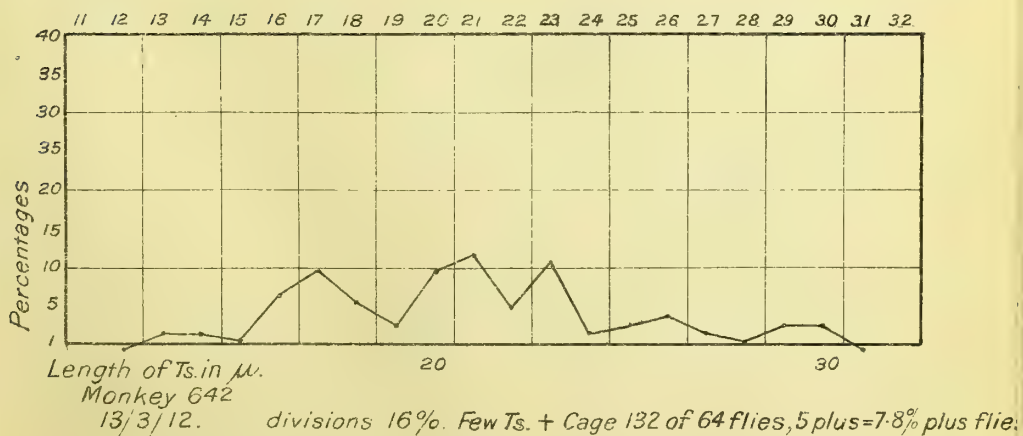
Ts. very scarce 20 drawn 1 div. = 5%. Cage (122), 57 flies 3 $\neq$ = 5.2% of flies.

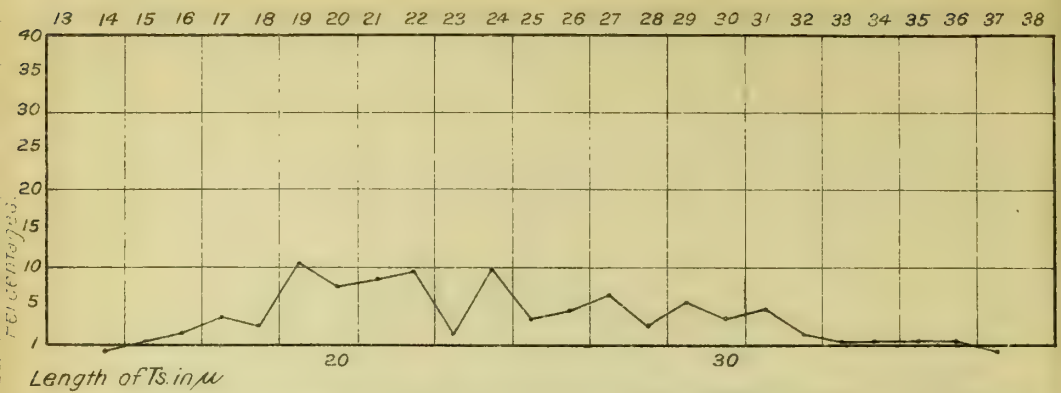


XX.

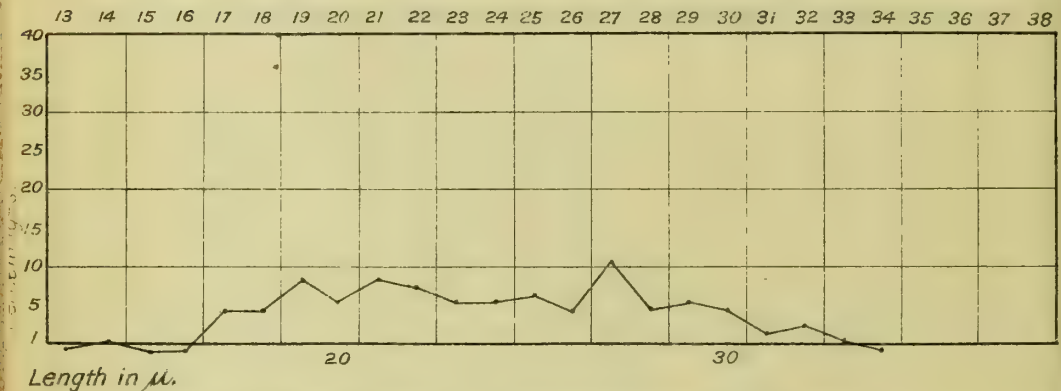


XXI.

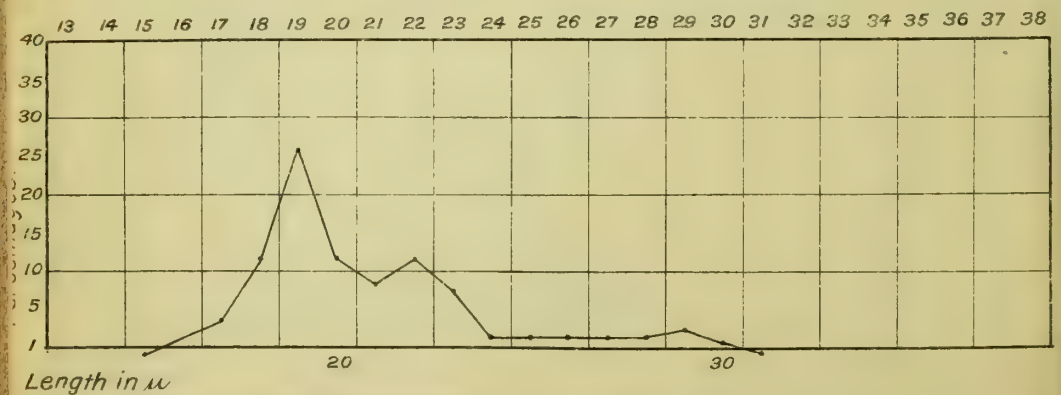




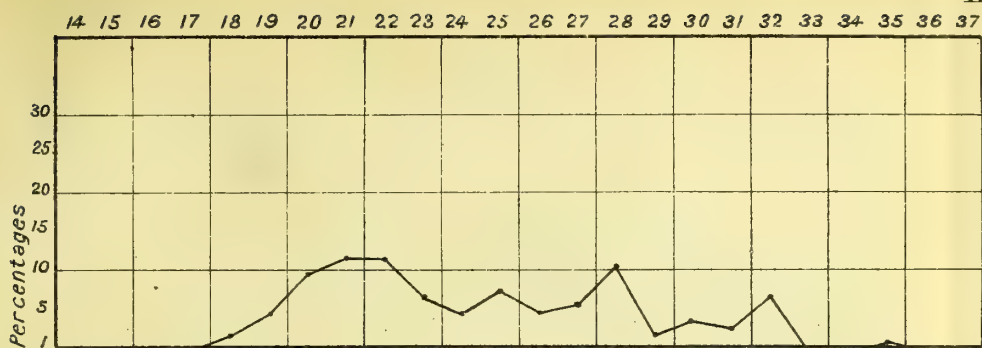
Monkey 642 divisions 26%. fair N° of Ts.++. Cage 134 fed, 78 flies, 6+ = 7.6% + flies.
14/3/12.



Monkey 642 divisions 24% Good numbers of Ts.++ to +++.
15/3/12. Cage 135 fed 75 flies, 6+ = 8% + flies.

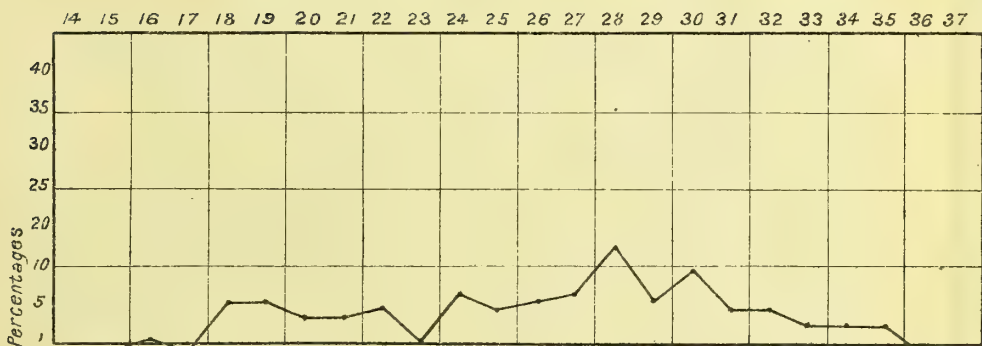


Monkey 642 fair N° of Ts.++. Cage 136 fed. 58 flies, 5 plus = 8.6% of + flies.
16/3/12. divisions 4%



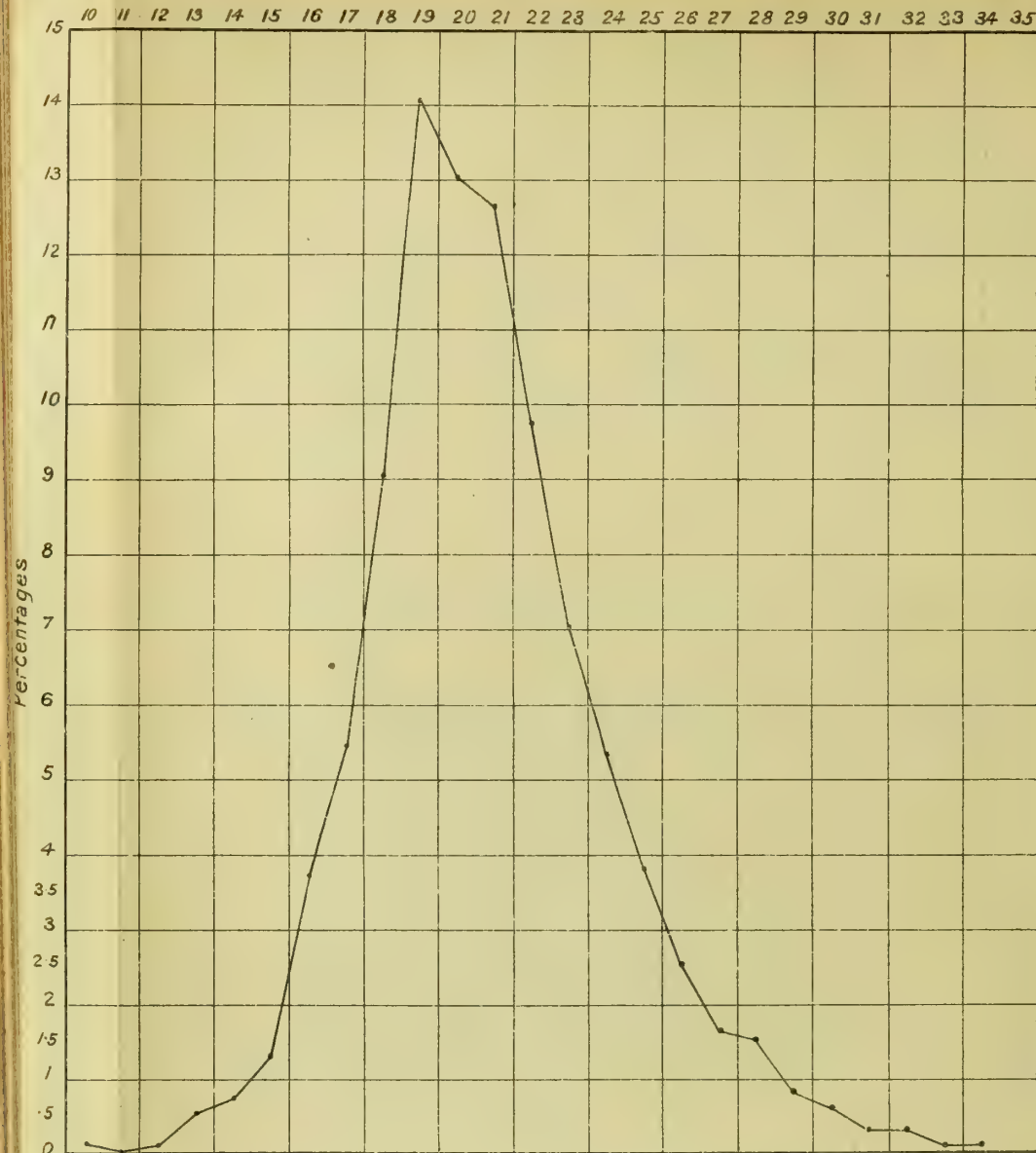
Length in μ 1st day of inf. div. 15% Ts. + to ++
 Monkey 631
 6/4/12.

Cage 148 fed 95 flies all negative.



Length in μ 1st day of infection divisions = 30%
 638
 9/4/12. Ts. numerous, ++ to +++

Cage 151 fed 129 flies, 4* = 3.1%.



Percentages	0.04	0.00	0.03	0.51	0.7	1.3	3.7	5.4	9.0	14.0	12.9	12.6	9.7	7.0	5.3	3.8	2.5	1.6	1.5	0.8	0.6	0.3	0.3	0.03	0.04
Actual N ^o	1	0	3	11	17	29	31	118	198	305	283	272	210	154	115	82	56	36	34	18	13	7	7	2	1
Length in M.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34

Chart of all non-dividing forms in Experiments. N^o of Ts = 2155.

13.—NOTES ON THE BEHAVIOUR OF A POLYMORPHIC TRYPANOSOME IN THE BLOOD-STREAM OF THE MAMMALIAN HOST.

By MURIEL ROBERTSON, M.A.

In a previous paper the conditions obtaining in the peripheral blood of monkeys infected with *Trypanosoma gambiense* were treated in some detail, and it was found that a certain sequence of forms recurred at irregular intervals, but that the nature of the sequence was extraordinarily uniform. This recurrence of certain types was termed the endogenous cycle, and it was pointed out that this development and the duration of the different phases were dependent on the inter-relation of two complex groups of factors, namely those involved in the reaction and general metabolism or the host and those involved in the similar processes of the parasites.

The main result of this work was to show that the well known polymorphism of *T. gambiense* was a growth and dividing phenomenon—the short forms, from their greater duration in time and their importance in the intermediate host, were termed the adult individuals; the intermediate types were shown to be growth forms and the long slender individuals were shown to be those about to divide. The products of division directly or indirectly produce the short forms.

As this interpretation was quite unsupported by the views of other workers, the earliest opportunity was naturally seized to test its value in the case of another polymorphic species of trypanosome. The trypanosome here discussed was forwarded to Mpumu by Mr. Montgomery of the British East Africa Veterinary Department. The species was tested in a number of ways by Dr. Lyndhurst Duke with a view to diagnosis; his results are above (p. 67).

The trypanosome is apparently nearly allied to *T. pecaui* but does not coincide with this species in certain important points, it was therefore very fully described in all its available manifestations and reactions but was left unnamed.

The species, which is morphologically very like *T. brucei*, is markedly polymorphic and is well suited to test the question at issue. Moreover, it was readily transmitted by *G. palpalis* so that the strain had the opportunity of experiencing the normal passages through a suitable insect host.

As in *T. gambiense* the numbers in the blood fluctuate but there are two slight differences in that the depressed periods are often very short—12 to 24 hours, and the tendency to temporary checks in the numbers during a general increase is more marked than in *T. gambiense*. The drop also may be more gradual and less dramatically sudden than in the species already investigated. Nevertheless depressed periods of 3 to 4 days occur at times just as in *T. gambiense* and perfectly smooth rises to a high numerical climax followed by a sharp drop are also not by any means rare occurrences. Briefly while as will be seen hereafter the morphological evidences of the cycle are perhaps even clearer than in *T. gambiense* the duration of the physiological phases are shorter and somewhat less sharply defined.

Method.

The method adopted in carrying out these observations is as follows. The films were taken from a infected monkey on consecutive days fixed by exposure to osmic vapour and immediately immersed in alcohol absolute. They were then dried in air and stained by Giemsa's routine method. The image of the trypanosomes were thrown on to a sheet of paper; by means of Abbe's drawing apparatus a line was carefully drawn down the centre of the image to the tip of the flagellum, this line being subsequently measured. The first hundred trypanosomes met with on the slide were drawn in this way including dividing individuals. This gives the percentage of dividing forms for the film in question. These dividing specimens were then neglected and the hundred non-dividing specimens were made up by drawing the requisite number of specimens, the types being taken as they came. The tables are all made up of the measurements of non-dividing specimens with the exception of table V which deals solely with division forms.

The actual measurement was done by Bruce's compass method. There seems to be an unfortunate tendency on the part of workers in England to complicate the methods of drawing and measurement beyond both their theoretical value and their practical application. All measurement however accurate leaves out of count the very obvious unevenness of all film fixation. There appears to be a difference of a minute fraction of a micron between the result of Bruce's very simple and readily applicable method and that adopted by the Liverpool workers. (See Fantham and Stephens, *Bull.* 36, and Bruce, *Bull.* 39.) The latter method taken in its entirety may be beyond criticism, but it is also entirely beyond the application of any worker out of Europe. No point of diagnosis between different species or of differentiation between the forms within one species turns upon a micron or even two microns one way or the other. For instance, as is well known, *T. evansi* and *T. brucei* can be differentiated upon the minimum measurements, the minimum measurement in *T. evansi* being about 5 microns greater than the corresponding measurement in *T. brucei*. But were the difference between these two measurements only 1 or 2 micron, no one would dare to use that character as a means of diagnosis.

One would therefore plead for a uniform rapid and simple method of measurement which would admit of large numbers of individuals being handled and which could be applied by colonial workers, who after all are the people who are seriously and frequently faced with the practical questions of species diagnosis in the ordinary course of their duties. Such a method, namely that of Bruce is already at hand and in use, and it seems a pity that it should not be universally applied. Measurement by itself tells only a very limited number of things about an organism, and there seems to be a danger of the biometrical handling of Trypanosomiasis falling into the familiar error of regarding the method as an end in itself out of proportion to its value as a means of obtaining information.

One point is made very clear by the results to be described in this paper, namely that the relative percentages of long, short and intermediate forms are of absolutely no diagnostic value whatever, these proportions change from day to day in direct correlation with the phases of the endogenous cycle. See Tables III. and IV.

I should like to mention in passing that one hundred non-dividing specimens happens to be a very satisfactory number as a sample of the daily condition. This is shown by the fact that two such samples taken by the same observer form the series of slides of one day, but drawn at an interval of a week gave in two instances averages differing only within .5 of a micron. A similar correspondence occurred on two other occasions when 100 individuals from two other series of homologous slides were drawn by two different observers. From the four independent cases of correspondence it seems reasonable to conclude that a 100 non-dividing specimens will give a fairly accurate account of the average length of the trypanosomes for that day and a fairly reliable picture of the general conditions. It is however clear that fluctuations in the distribution of lengths will not always be detected from the averages. The weakest part of the method used is the absence of really precise estimate of the numbers present on each day.

The following table (Table I.) gives the distribution of length of 100 non-dividing specimens from each of 6 consecutive days, the monkey (830) was infected by laboratory-bred fly carrying the trypanosome in question and Sept. 27th, 1912, was the first day upon which trypanosomes were to be seen in the peripheral blood. The percentage of dividing specimens found on each day and the numerical symbol of the infection are added in the right hand columns.

Dividing specimens are not included in the measurements of the Tables I., II., III., and IV.

Monkey 830.

Table I.—Distribution of Trypanosomes of various lengths.

Date.	Length of Trypanosomes in Microns.																																			Per cent. of divisions on date.	Numerical symbol.
	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.															
27.9.12					2	3	7	1	9	9	11	10	14	6	4	6	7	5	3	2			1	Per cent.											27	+ to ++	
28.9.12	1		1	2	5	3	7	11	18	14	12	5	10	4	3	2								18											18	++	
29.9.12	1	3	10	19	18	13	4	8	7	7	4	1	3	2										4											4	+	
30.9.12	7	25	18	23	15	6	3	1	2															4											4	+	
1.10.12	1	3	10	10	13	16	10	10	8	10	2	2	3	1			1							10											10	++	
2.10.12	1	1	6	8	18	6	10	8	11	11	1	3	3	1	4		3	1						9											9	+++	

Table II. is the corresponding account of the distribution of lengths of 100 non-dividing specimens taken daily from monkey 821 throughout 16 consecutive days. The monkey was inoculated on Aug. 15th and first showed trypanosomes four days later.

Tables III. and IV. give the same data as Tables I. and II., but considered from a different aspect. The percentages of long, short, and intermediate trypanosomes are given for each day, the average length and the percentages of dividing individuals. The division into the three types is of course arbitrary, a specimen of 15 microns is obviously short and one of 27 microns is equally obviously long, but the actual lengths chosen as the dividing points are merely convenient signposts in the series. These tables show at once the manifest uselessness of the relative percentages of types as any sort of a guide in diagnosis.

Monkey 830.

Table III.

Date.	Per cent. of specimens between 12 and 18 m. inclusive.	Per cent. of specimens between 19 and 23 m. inclusive.	Per cent. of specimens above 23 m.	Average length.	Per cent. of divisions on date.	Numerical symbol.
	Per cent.	Per cent.	Per cent.	m.	Per cent.	
27.9.12	2	29	69	25.4	27	+ to ++
28.9.12	9	53	38	22.7	18	++
29.9.12	51	39	10	19.4	4	+
30.9.12	88	12	—	16.3	4	±
1.10.12	37	54	9	19.7	10	++
2.10.12	34	46	20	20.9	9	+++

Monkey 821.

Table IV.

Date.	Per cent. of specimens between 12 and 18 m. inclusive.	Per cent. of specimens between 19 and 23 m. inclusive.	Per cent. of specimens above 23 m.	Average length.	Per cent. of divisions on date.	Numerical symbol.
	Per cent.	Per cent.	Per cent.	m.	Per cent.	
21.8.12	0	26	74	26.7	20	++
22.8.12	9	16	75	26.8	24	++ to +++
23.8.12	25	36	39	22.1	22	+
24.8.12	76	21	3	16.9	6	±
25.8.12	31	49	20	19.3	29	+
26.8.12	16	40	44	22.3	16	++
27.8.12	27	41	32	21.5	9	+++
28.8.12	57	37	6	18.5	8	++ to +++
29.8.12	76	21	3	17.1	3	+ to ++
30.8.12	79	17	4	17.0	12	++
31.8.12	38	44	18	20.0	20	+++
1.9.12	54	40	6	18.0	12	+++
2.9.12	48	34	18	19.5	16	++
3.9.12	78	14	8	16.8	10	++ to +++
4.9.12	83	15	2	16.5	6	++ to +++
5.9.12	15	34	51	23.3	37	+

Table V. gives the distribution of length for 250 dividing individuals drawn from the infection of 821 studied above. The results for all the days are combined.

Monkey 821.

Table V.—Distribution of length of Dividing Trypanosomes.

Length in m.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	Total Number.
Number of Trypano- somes of length.	1	2	3	11	20	36	38	23	33	30	11	16	9	8	1	5	3	250

Two points must be borne in mind in considering these tables.—

(1) The trypanosomes are living in an active and at times even a definitely hostile environment and therefore do not have it all their own way.

(2) The percentage of forms about to divide will be greatest at the beginning of a rapid rise in numbers and will naturally diminish in frequency as the numerical height for any individual revolution of the cycle is reached, and this relative diminution in the forms about to divide will of course precede somewhat in time the actual diminution in the forms in division. Equally the percentage of forms about to divide will be lower in comparison to the forms in division in a slow rise. To put this briefly, the time factor in the process of multiplication is bound to affect the relative balance of the different types.

From Tables II. and III. it is clear that there is a definite general correlation between a high percentage of long forms and a high percentage of divisions thus to pick out a few typical cases: In Monkey 830 on September 27, 1912, there were 69 per cent. of long forms and 27 per cent. of divisions, in Monkey 821 on August 22, 1912, there were 75 per cent. of long forms and 24 per cent. of divisions, and in the same monkey on September 5, 1912, there were 51 per cent. of long forms and 37 per cent. of divisions. There is equally a clear correlation between a high percentage of short forms and a low percentage of divisions, and moreover Table III. of Monkey 830 shows strikingly how here as in *T. gambiense* the depressed period immediately after the fall in numbers is characterised by the presence of short forms; thus on September 30, 1912, the day in question there were only 4 per cent. of divisions and 88 per cent. of all the parasites were below 19 microns, the longest form present was 22 microns and there were only two specimens of this length. That is to say that just as in *T. gambiense* the survivors who weather the untoward conditions which carry off the majority of the parasites are drawn from among the short forms.

Now it may also be seen by looking at Table V. and by a study of the morphology of the trypanosomes that division is not found in specimens below 20 to 21 microns in length, and as a matter of observation, the earliest forms in which any sign of division can be detected are definitely long forms. From the evidence of the tables it seems just to conclude that the long forms are those about to divide and that they produce the short forms, in some cases directly, in others probably upon the intervention of another or several other divisions. A study of late division stages as well as a mere observation of the revolution of the cycle as shown by the measurements lead to this consideration.

The fact that the short forms, both in *T. gambiense* and even more markedly in the present trypanosome, are very bulky relatively to their length and body surface suggests that a certain amount of actual retraction may occur after division; and further that as well as mere growth there is a definite lengthening of the parasite at the expense of the amount of protoplasm contained within the periplast at the time when division is about to take place. In an organism whose nutrition is absorbed entirely through the general surface of the body by what appears to be a process of diffusion, the obvious increase in of the surface in relation to the bulk of protoplasm in a long slender individual suggests a possible clue to the rationale of the lengthening of the body form before division. The forces at work in a dividing cell and the conditions that call them into action are still exceedingly obscure, but it may safely be assumed that the period of division is one of heightened cell activity. The increase of the body surface, whether due to the need of increased nutrition or more active respiration is pretty clearly correlated in these trypanosomes with the peculiar physiological activities of division. I would like to point out that the above suggestion does not touch upon the question of the stimulus to division in the case of these trypanosomes, but merely implies that in response to that stimulus whether internal or external there is an increase of the body surface. It is clear that an increase in the body surface is not a necessary condition of trypanosome division *quâ* division as is instanced by the behaviour of the same organisms in the fly nevertheless it must be remembered that the physiological environment of a trypanosome in a highly oxygenated blood stream must be very different from that obtaining in the alimentary tract or salivary fluid of the fly.

It will be well in conclusion to run briefly through the phases of Monkey 821 as illustrated in the Tables II. and III. to show the nature of the cycle.

The 21st and 22nd of August are days of a rapid increase in the absolute numbers and are characterised by a high percentage of long forms. The 23rd shows a sudden drop in the numbers, in spite of an obvious attempt at further multiplication as is evidenced by 22 per cent. of dividing specimens. The 24th is a typical instance of a depressed period, the short forms number 76 per cent. Trypanosomes are very scarce, and two 3 by 1 films had to be searched for many hours to obtain the number requisite,

only 3 per cent. of long individuals are to be seen, and the divisions are equal to 6 per cent.

From the 25th on there is a slow and steady rise for several days, the 25th itself shows 29 per cent. of division and a large percentage of intermediate forms, 49 per cent., though only 20 per cent. of long forms. The long forms increase on the 26th though the divisions are beginning to tail off a little. The rise continues slowly till on the 27th the trypanosomes are very numerous, on the 28th the advance in the numbers is arrested and the types are preponderatingly short and intermediate, the proportion of division is low, this is a typical high level period where the cessation of division is characteristically correlated with the marked diminution in the number of long forms. On the 29th the divisions drop still further and there is an absolute diminution in numbers though it does not amount to a serious clearing out of the trypanosomes from the peripheral blood. By a curious coincidence, although the detailed distribution is different and the absolute numbers are much higher, the relative percentages are identical with those of the 24th, which as we have seen was a typical period of depression. On the 30th the divisions met with are more numerous, but there has only been a slight increase in numbers in the interval and the short forms still preponderate very much. On the 31st a rise from the level of the last two days is definitely underway and just as before the intermediate forms increase as also the long forms. On the 1st of September the rise is maintained but not increased, there is a setting back towards a very high proportion of short forms, and between the 1st and 2nd there has been a slight drop in the numbers, but the increase of the long forms suggests that the check is not to be of prolonged duration. By the 3rd there has been an actual though not very great increase in numbers, this advance is maintained but is not pushed further on the 4th when another typical climax is seen; that is to say a period of high absolute numbers, few divisions, few long forms, and a quite overwhelming majority of short forms. The following day is particularly interesting as it shows a condition diametrically opposed to the one which immediately preceded it. Between the 4th and 5th there has been a very marked drop in absolute numbers, but by the time the sample was taken there is already a very serious attempt on the part of the trypanosome infection to recover the lost ground; and with relatively low absolute numbers there are 37 per cent. of divisions and 51 per cent. of long forms. This attempt at a rapid rise, which looked as though it would resemble the progress of affairs on the 21st and 22nd of August was in this instance frustrated, and the 5th September was followed by a depressed period of four days in which there were very few trypanosomes present, and these were of the short type.

It seems quite impossible to escape the conclusion that the long forms are the individuals about to divide, that they produce the short forms which are the most stable type and which have almost exclusive possession of the field the moment the divisions are markedly reduced, the intermediate forms being simply the

merging of the short into the long types. The irregularity of the cycle should be noted, the variability depending on the complex interaction of the resistant forces of the host and the aggressive capacity of the parasites. It is pretty clear that such a cycle of changes must suggest an alteration in the current method of diagnosis by measurement and explains many of the puzzlingly divergent results obtained by highly competent observers.

Mpumu,

November 4, 1912.

14.—NOTES ON THE LIFE-HISTORY OF *TRYPANOSOMA GAMBIENSE*, WITH A BRIEF REFERENCE TO THE CYCLES OF *TRYPANOSOMA NANUM* AND *TRYPANOSOMA PECORUM* IN *GLOSSINA PALPALIS*.*

By MURIEL ROBERTSON, M.A.

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[PLATES 2-7.]

INTRODUCTION.

Among the mammalian trypanosomes carried by *Glossina palpalis*, *T. gambiense* may be taken as an example affording, as it were, a basis for the study of the group. In many ways the conditions are very clear, and no very serious difficulties on the score of technique or scarcity of parasites are met with at any point in the *Glossina* cycle.

In this paper I have considered the cycle of *T. gambiense* in detail, and have treated *T. pecorum* and *T. nanum* comparatively and much more briefly. I am indebted to Dr. H. L. Duke for the material of *T. pecorum* and *T. nanum* dealt with.

The material of *T. gambiense* was derived from a large number of experiments carried out in the Mpumu Laboratory in 1911 and 1912. The stages in *Glossina palpalis* were obtained from the study of the conditions in more than 200 infected flies, which were killed or died at different periods of the cycle. The advantage of so large a number is obvious, in that it eliminates casual variation. A considerable number of different strains of *T. gambiense* were used; thus, two separate strains derived from wild fly caught in Chagwe, a strain derived by direct injection into a monkey from an antelope infected with a human strain, this same strain after transmission by flies to clean monkeys, antelope strains of human origin passed by fly to clean monkeys, and a number of indifferent Uganda strains kept going among the Mpumu monkeys by means

* Reprinted from the Philosophical Transactions, Ser. B, vol. 203, p. 161.

of *Glossina palpalis*, all served at one time or another as infecting material for the flies employed in the experiments.

I need not go into the very important question of the diagnosis of the strains derived from the Lake-shore flies, as that has already been dealt with by Dr. H. L. Duke in a separate paper.

In all the experiments, the flies used belonged to the Uganda variety of *Glossina palpalis*,* and were hatched out in the Mpumu Laboratory from pupæ brought from Damba Island in the Lake. Experiments had been carried out by Captain Fraser and Dr. Duke with several hundreds of these laboratory-bred flies, to ascertain if hereditary infection with flagellates of any kind occurred. The results were invariably negative. A similar conclusion had been reached by workers in other parts of Africa, notably by Dr. F. Kleine and his colleagues in German East Africa. No further experiments were made, therefore, in regard to this point, which seems now to be established beyond reasonable criticism.

Methods.

The method adopted in the study of the fly cycles was as follows: The newly hatched flies were starved for about 24 to 36 hours, and were then fed on the infecting monkey once, or in some cases twice. The infecting feed was the first blood ingested by the flies. After the infecting feed, the cage was starved for one or two days, and thereafter fed on clean monkey's blood every second or third day. Daily feeding is not essential to the welfare of the *Glossina*, and does not appear to occur in nature.

Late or provedly infective boxes were fed as a rule on cock's blood, but these cages were not used for gut stages, in order to eliminate all chance of accidental confusion with the trypanosome proper of the cock (*T. gallinarum*), which, as has been shown by Dr. H. L. Duke, not infrequently undergoes development in the *Glossina*.

Dissections were made in a drop of physiological salt-solution. The trypanosomes were studied both in the live state and in fixed and stained preparations. Unfortunately, trypanosomes do not live for any length of time in a normal condition outside the body of the fly, even when the fly has been dissected in monkey's serum.

The preserved material was fixed while wet by dropping the cover-slip film side downwards on to Schaudinn's corrosive-alcohol fixing solution. The preparation was subsequently stained by Heidenhain's iron hæmatoxylin method. This treatment of the films gave excellent results, and affords a more accurate account of the parasites than that obtained from the Giemsa method.

I. ENDOGENOUS CYCLE IN THE VERTEBRATE.

The life-history of *T. gambiense* falls naturally into two parts, namely, the endogenous cycle in the vertebrate, and the exogenous cycle in the transmitting host. Certain aspects of the phases in

* See 'Sleeping Sickness Bulletin,' No. 38, vol. 4, for Dr. R. E. McCONNELL's account of *Glossina fuscipes* in Uganda.

the vertebrate have formed the subject of a previous paper, and they will therefore be treated very briefly here.

The course of the endogenous cycle as a whole is as follows: the description is drawn from the study of the conditions as found in monkeys (a species of *Cercopithecus*). In monkeys the incubation period is usually seven days. The organisms are very scarce in the blood at first, but increase rapidly in number. The multiplication takes place in the circulating blood, and intracellular multiplicative forms have not been observed at any time in the lung, liver, or spleen.

The forms undergoing schizogony in the lung, described by Vianna,* have never been seen, even in the earliest days of the infection in monkeys. Vianna's results are derived from guinea-pigs and white rats, and, as far as can be seen from the account given, there are no details concerning the origin of the strain used.

Intercellular forms have, however, been found in the lung and liver at certain periods, as will be seen hereafter, but they are involution-forms, and possibly latent forms, and no sign of multiplication has been observed.

The trypanosomes in the blood vary, as is well known, considerably in length and breadth, and the species shows the polymorphism characteristic of that set of trypanosomes, usually referred to as the "*brucei* group." From a long series of experiments, described in detail in the paper mentioned above, I have been led to the following interpretation of the cycle in the vertebrate. As here given, the account appears as a set of unsupported statements, and the reader must be referred to the earlier paper for the evidence upon which they are based:—

The short forms, 13 to 20 microns in length, are the "adult" blood type—this term being used to indicate that the form in question has the longest duration in time in the endogenous cycle, and appears to be the type from which the individuals capable of carrying on the cycle in the invertebrate host are derived. The blood of a monkey is infective to *Glossina* only so long as this form is present in sufficient numbers and in an appropriate physiological state—i.e., not suffering from the exhaustion which seems to overtake the flagellates at certain times in very numerous infections. From these short forms there arise by growth the intermediate individuals, which are an ill-defined and quite artificial group, chiefly to be recognised by the fact that they have increased in length and have a longer free flagellum, but are of much the same average breadth as the short forms, namely, 2 to 2.5 microns. The long slender forms are derived from the intermediate type, and are simply the next step towards the division forms, which are the culminating stage of this whole process. The long slender forms are the individuals about to divide. It is incorrect to speak of three types of trypanosome; strictly speaking, it is a somewhat arbitrary attachment of names to the different stages by which the short forms proceed to division. The products of division give rise once more, directly or indirectly, as the case may be, to the adult type.

* 'Bulletin,' No. 38, vol. 4, p. 238.

Another feature equally well known in the history of *T. gambiense* is the fluctuation in the number of trypanosomes to be seen in the blood. This has also been treated exhaustively elsewhere; it suffices to say here that a complex of circumstances, occurring at quite irregular and apparently incalculable intervals in the blood of an untreated vertebrate, brings about the disappearance of the vast majority of the parasites from the peripheral blood. The duration of the depressed period is very variable, and the reappearance of the flagellates is always accompanied by division in the peripheral blood. The percentage of divisions bears, moreover, an obvious relation to the rapidity of the rise in number. An important feature of the depressed period is that, from the completion of the fall in numbers, which is usually rapid, until the first beginnings of the following rise, the blood is infective to fly, and the few surviving trypanosomes are of the short type.

The mechanism of the destruction is difficult to follow, but a certain amount of trypanolysis seems to occur in the blood. The liver and lungs have shown, in the case of a very teeming infection investigated at this period, both degenerative forms and what one is inclined to call involution-phases (Fig. 39B). The liver of this monkey (633) showed many rounded-off individuals, possessing a trophonucleus and a kintonucleus, but no flagellar apparatus. These forms seemed for the most part to be between the cells, and only in a few instances to be inside the cell—but this is a point very difficult to determine accurately in smear-preparations. In addition to the rounded-off specimens, there were many trypanosomes of degenerate appearance. It is impossible to state what the fate of the rounded individuals may be. The infectivity of the blood during the depressed period, and the failure to find multiplicative intracellular stages, coupled with the gradual nature of the rise and the high percentage of dividing individuals in the peripheral blood during this increase, are all arguments against the form under discussion playing any serious part in the reappearance of the trypanosomes. These forms were not to be found in two late and chronic infections investigated, nor were they observed in a rather interesting case of an infection which had been a very teeming one, and which had just begun to take on the more chronic characters. This monkey died apparently from exposure during a severe storm, in which the animal had refused to stay in its box. It was observed when just dead, and the smears were taken at once. At the time of death, the infection was showing its first longer period of low numbers; it had been in a swarming condition only 10 days prior to the taking of the films. The infection had, of course, shown the usual short depressions, but these had been less frequent than is typical of the progress of affairs in monkeys.

It is impossible to dismiss altogether the consideration that the rounded forms may be latent individuals (capable of multiplication and further activity) lying in small numbers in the liver and lung, though their persistence in the latter seems less probable. Nevertheless, the three cases just cited do not bear this out, and the evidence, so far as I have been able to see, is all in favour of the rounded forms being destined to destruction. The point must,

however, be borne in mind, as the possible survival of latent forms, however scarce, in contact with or enclosed in the cells of any part of the body is exceedingly important from the therapeutic point of view.

The question of a sexual interpretation of the polymorphism of *T. gambiense* has very frequently been raised, and has, indeed, already passed into the nomenclature adopted by many workers, so that one is constrained to give it a consideration it does not really deserve on the strength of its merits as a scientific hypothesis. The interpretation put upon the appearances is that the long slender forms are males, the short forms females, and the intermediate individuals are indifferent, or simply non-sexual forms. This is based primarily on a vague analogy with the life-cycle of the malarial parasite, and on the supposition that the long and short forms play the part of gametes or gametocytes in the transmitting host.

All the evidence brought to light by the study of the endogenous cycle is in absolute opposition to the sex-interpretation above sketched, and it may be pointed out that it is direct evidence of a nature easily observed by a careful study of the successive stages of the blood-cycle. The results of a long series of feeding experiments carried out on consecutive days with *G. palpalis* have further shown that it is on the presence of the short forms that the infectivity of the blood depends. It must be noted here that the distinction between the broad and slender forms is merely a physiological one, and is apparently entirely in respect to the process of division. The reason why it is from among the broad forms that the survivors in the fly are drawn is most probably that they are in a more stable condition, and therefore more capable of resisting the change of environment. Dismissing, then, the division of the long and short forms in the blood of the vertebrate into sex-categories, there remains to be considered a sexual differentiation among the short forms. I have not been able to detect any morphological distinction that could reasonably be attributed to sex. There is a certain amount of variation among the short forms in length and breadth, but it is not marked, and the nuclear features are very uniform. If the short forms do consist of male and female gametes or gametocytes, the differentiation is not expressed morphologically.

Cytology of T. gambiense in the Blood of the Vertebrate.

To consider very briefly the finer structure, and the process of division of *T. gambiense*, as studied on blood-films fixed by Schaudinn's corrosive-alcohol solution and stained by Heidenhain's iron-haematoxylin method. The body-form of trypanosomes is too well known to require description, and the species in question shows the typical relations. The kinetonucleus is small in size, and shows the usual two aspects, being either rounded or slightly rod-shaped. Close beside the kinetonucleus, at the actual origin of the flagellum, may be seen a small granule, the blepharoplast proper, or basal granule. The specimens derived from monkeys show a

finely granular protoplasm, and larger inclusions are very rarely seen. The current view of the trophonucleus of *T. gambiense* is derived from dried Giemsa preparations, and bears only the slenderest relation to the real state of affairs. It is always figured as a large round or oval mass of granular chromatin.

With good illumination and a high-power system, any patient observer can see the nucleus in favourable specimens in the live state. This is, of course, more easily achieved in the larger species; in the trypanosomes of fishes and reptiles, an immersion-lens is not essential. The live picture is in all cases that of a circular, slightly refractile object, lying surrounded by a clear halo. The material fixed by the wet method, and never dried in air at any period during the preparation of the slide, shows conditions very closely resembling the live picture, and utterly different from the version given by the usual Giemsa method. All fixation is probably a choice of error, but it stands to reason that if a delicate and highly flexible organism occupying three dimensions is swiftly flattened out into two, quite regardless of its attitude at the time, disasters are liable to occur. In dry preparations the nucleus has simply been burst. There is nothing wrong with this method so long as the observer remembers that he has destroyed this important structure, and does not proceed to describe it as though he had respected its integrity. The criterion of fixation must be the relation of the fixed material to the live picture, and, judged by this standard, the wet fixation and passage into some mounting fluid is greatly to be preferred. The criticisms that may be urged against the wet method are slight shrinkage of the protoplasm of the body, and the fact that, as the attitude in three dimensions has been very closely preserved, it is almost impossible in many cases to get an accurate microscopic measurement of the length of the animal. The ordinary methods of measurement supply no means whereby the elevation towards the eye of the observer can be accurately estimated in the case of small objects of irregular shape. Another drawback to the wet method is that all the parasites in a drop of fluid may not adhere to the cover-slip; it is clear that in certain kinds of work this is a very serious disadvantage.

The trophonucleus is of a type very common among flagellates, and is characterised by a large central karyosome, in which almost all the chromatic material is concentrated. This is surrounded by a clear space, which is in turn bounded by a faintly staining membrane or nuclear boundary—the word membrane is convenient, but its use here is not very clearly justified. Very delicate strands radiate out from the karyosome to the membrane; they are not always very clearly visible, but are to be made out in the vast majority of specimens. This condition of the nucleus is extraordinarily constant; practically no variation is found in any of the non-dividing blood-stages, and it is also found to persist through a large portion of the cycle in the fly. An important change does, however, take place in the latter part of the *Glossina* cycle; this will be noticed and discussed in treating of the forms in question (see Plate 2, Figs. 1-4).

Division of the Trypanosomes in the Blood of the Vertebrate.

This process resembles very closely the division in the fly cycle, where the details are much clearer, so, to avoid repetition, I shall only draw attention to the most important features. The first sign of division is the doubling of the kinetonucleus. The behaviour of the blepharoplast is not clear, but Fig. 6 suggests that it has the centrosomic function to be noted in the division in the fly cycle. The flagellum splits, but never throughout the whole length; it becomes free about two-thirds of the way down, and the flagellum of the daughter-individual is usually shorter than that of the parent. The trophonucleus shows one very interesting feature before division. When only the kinetonucleus and the flagellum have as yet begun to show signs of re-duplication, two very well marked dark granules are to be observed on the membrane at opposite poles (Figs. 5 and 6); they are generally joined by a fairly thick line to the karyosome. The other strands passing from the karyosome to the membrane persist for a time, but disappear when the division-figure begins to be formed. The two granules just mentioned apparently play the part of centrosomes. I have not been able to trace the exact place of origin of the granules. They seem certainly to be intranuclear, but I am quite unable to say if they arise from the karyosome. The division-figures are shown in Figs. 5-8, Plate 2. There is no equatorial plate.

II. EXOGENOUS CYCLE IN THE FLY.

(1) *Conditions Obtaining in the Alimentary Canal of G. palpalis in Relation to the Development of T. gambiense.*

In considering the exogenous cycle, a few points bearing on the habits, etc., of the *Glossina* must be touched upon in passing.

The general anatomy of *Glossina palpalis*, and the structure of the proboscis, and the relations of the salivary glands, are so well known from the excellent work of Minchin, Stuhlmann, and others, that I shall not touch upon them. Following the practice of the Uganda Sleeping Sickness Commission of 1908, I have, in considering the infected flies, divided the portion of the gut which lies between the proventriculus and the origin of the Malpighian tubules, and which constitutes morphologically the mid-gut, derived from the embryonic mesenteron, into three parts, namely, the anterior or thoracic, the middle, and the hinder intestine.

From the dissection of a considerable number of wild tsetse from the Lake-shore, caught only three or four hours previous to examination, it appears that, under natural conditions, the majority of flies certainly do not feed daily. In most cases the whole gut is empty of food-material, except for a small quantity of pale greenish, completely digested fluid in the portion of the gut just anterior to the Malpighian tubules. This condition indicates a fast of at least two or three days, and possibly of a very much longer duration; in captivity, flies will live from nine to twelve days without food if the conditions of moisture are suitable. A large number of experiments of long duration were

conducted, in which the cages were starved for two complete days between each feed, but there was nothing abnormal in the number of deaths. The fact that wild flies do not feed at such very close intervals of time as seems to be implied from the usual laboratory treatment of the flies has, as will be seen later, a somewhat important bearing on certain parts of the cycle.

The actual mechanism of feeding calls for attention, in that it affects the course of development. The sucking-stomach or crop leads out of the ventral side of the proventriculus by a long duct; in a full feed, the gut, up to a short distance behind the proventriculus, and the crop are both filled with blood. As digestion proceeds, the blood in the gut diminishes, and that from the crop is gradually passed, first forward along the duct, and then back down the gut. In a smaller feed, the gut may be completely filled up to the proventriculus without blood being taken into the crop at all. Flies are willing to feed before digestion is anything like complete, and it is important to know what occurs when the new blood is ingested. The experiments were made by feeding flies alternately on monkey blood and cock's blood.

The very first feed taken by a fasting *Glossina* (24 to 48 hours old) is usually a full feed, pretty well filling both crop and gut. The next feed, ingested 60 to 72 hours later, may behave in one of the following ways: The new blood may entirely replace all the material of the first feed from both crop and gut alike. In a large number of cases, however, some of the first feed is retained in the crop, and the second feed is taken in on top. In these cases one may also often find that the new blood has replaced all the first feed from the gut. There is thus a mixture in the crop, while the gut is, at the conclusion of the feed, full only of the new blood. In cases where digestion has been slow, the blood in both the crop and the gut may be mixed. Another state of affairs occurs, in which none of the new blood is taken into the crop, although the gut may be filled with the material of the second feed; moreover, in some cases, part of the original blood may be retained in the crop unmixed, and apparently unaltered, for as much as 10 days or more, although frequent feeds have intervened. A case of this kind was also observed in an experiment conducted by Dr. Duke and Captain Fraser.

The rôle of the crop in the economy of the fly appears to be simply that of a store-chamber; no digestion takes place there, and, in flies that have been allowed only one feed, and are thereafter starved entirely, one may find blood, the elements of which are optically unchanged after as much as 12 days. The crop can be completely emptied, and is, indeed, very often found in that state. It is also very frequently found, as is well known, to contain a bubble of gas.

Now, if a newly hatched fly has received trypanosomes with its first feed, it is interesting to see what has been the result of the subsequent clean feed.

This experiment was carried out with small groups of flies in glass jars. They were fed on blood containing numerous parasites; the greatest care was taken to see that all the flies had fed, there being, of course, no difficulty in ascertaining by inspection

whether a fly has fed or not. The clean feed was of cock's blood. The results here noted are partially drawn in addition from some jars fed only once, and killed after a suitable interval without a second feed. Further evidence is derived, moreover, from the many early dead flies examined in the course of the general mass of experiments.

(1) The trypanosomes may be digested and disappear from the whole alimentary canal of the *Glossina* during the digestion of the first feed, *i.e.*, during the first 50 to 72 hours, without the intervention of a second feed.

(2) The trypanosomes may survive in the gut in small numbers, but disappear during the early stages of digestion of the new blood.

(3) The trypanosomes may survive and multiply in the gut in the blood retained from the first feed, although a second feed has been taken in on top.

(4) Trypanosomes may survive and develop in the crop for as much as 12 days, provided the crop has never been entirely empty of blood during that period. The gut of these flies may often show no signs of trypanosomes. The trypanosomes in the crop seem unable to survive the emptying of this organ, and there is never a permanent infection in this situation.

(5) The trypanosomes may persist in greater or less numbers in the gut and in the crop of the same fly.

(6) The whole material of the first feed may be displaced from the gut by the second feed, and the trypanosomes still persist. The crop in these cases was either empty or filled with the new blood.

To consider which of the above conditions are likely to lead to an infected fly:—

(1) and (2) may be dismissed at once as negative.

(3) is a state of affairs that leaves the issue doubtful; if the trypanosomes are sufficiently established not to be swept out when the original blood is digested, a positive infection probably results.

(4) seems to suggest a negative result, as the condition of the gut implies that the trypanosomes are being digested and disposed of as they pass out from the crop into the gut.

(5) is a doubtful condition, almost identical with (3), though the chances of a positive result are obviously rather more favourable.

(6) seems to be pretty definitely a condition that will lead to a permanent infection of the fly. Once parasites are fairly well established in the new blood, the rate of multiplication is such that the chance of destruction at the next influx is small, and they have obviously been capable of withstanding the mere force of digestion by itself.

Clearly, two factors come into play here in the question of the establishing of an infection in the *Glossina*. First, the capacity of the trypanosomes to withstand the digestive processes; (1) and (2) show that this is not a property possessed by all the trypanosomes. The second factor is the clearing out of the trypanosomes

by the new feed; that this is a very potent agent in the production of negative flies is shown by the very much higher percentage of infected flies found up to the 12th day in experiments where the flies had only one feed, and that the infecting one. These experiments were carried out with controls, fed every two or three days in the usual way; the starved flies lived up to about the 10th or 12th day. Out of 103 starved flies, 22 showed trypanosomes between the 6th and 12th day. Neglecting those flies which showed trypanosomes only in the crop, there were 16 left, which showed apparently well-established, though not very numerous, infections in the gut, *i.e.*, 15.5 per cent. This is a very remarkably high percentage for the ordinary Uganda strain of *T. gambiense*. Under ordinary feeding conditions, the strain used for the starvation experiments just quoted produced 3 per cent. of + flies.

To quote a typical experiment. Jars Nos. 16, 17, and 18, each containing 15 flies, were fed on the same day and within a few minutes of each other on one monkey. Jars Nos. 16 and 17 were fed every third day, and dissected on the 22nd and 28th day of the experiment respectively; only one out of the 30 flies showed trypanosomes, and that was an individual which had died and been dissected on the second day after the infecting feed. Jar No. 18 was starved outright after the infecting feed; between the 9th and 12th days three flies showed trypanosomes in the gut, and three showed trypanosomes in the crop—altogether, 6 out of the total 15 showed trypanosomes. It must, however, be noted that the trypanosomes may disappear entirely from all the flies in a starvation experiment, and fall under the same heading as negative experiments in general.

To obtain therefore a clear idea of the elements that come under observation in the confusing early days of the cycle in the alimentary tract of the fly, it must be borne in mind that there are several conditions which may show trypanosomes persisting.

(I) Mere persistence without multiplication. Here the parasites undergo the usual slight alteration in shape, to be discussed later, and degenerating specimens are also to be seen. This condition is not found after 72-84 hours, and is chiefly to be observed when the trypanosomes were numerous in the infecting blood.

(II) Cases where the trypanosomes persist in small numbers in some part usually of the mid- or hinder intestine and multiply, but are, nevertheless, unable to establish themselves permanently. These are mostly cases where the apparently adverse conditions of the new feed come upon the trypanosomes before they are able to withstand them. In this state dividing and degenerating specimens are to be met with as well as numbers that do not suggest either condition. The individual types will be treated later. Persistence and quite normal development may occur, as has already been stated, in the crop, and continue till the 10th or 12th day, after which time I have not observed trypanosomes in this situation until they reappear there occasionally at the end of the cycle. This persistence and development in the crop is very interesting and important for two reasons: first, in that it obviously allows of several chances of infecting the gut, as all

the material in the crop, including the trypanosomes, is passed backwards down the alimentary canal. In the second place, it shows that the stimulus to development in the fly is not dependent upon the digestive action of the gut fluid upon the blood, *i.e.*, neither the digestive fluid nor its action upon the blood are essential factors in setting off the developmental processes.

(III) The third case found in the early days of the cycle is where the conditions admit of trypanosomes persisting and multiplying with sufficient vigour to establish themselves permanently in the gut. This may occur at any part of the middle or hinder intestine. I have never observed what one could with justice call established trypanosomes in the anterior intestine at an early period. This third condition leads to the production of a positive fly.

Out of a study of all these circumstances it may be deduced that, where no serious attempt at multiplication is made on the part of the trypanosomes, we have an expression of the incapacity of the parasites, and that we are dealing with a negative condition, that is to say, the large majority of the individual flagellates ingested are not in a fit state to carry on the cycle. This condition of the flagellates is, as has been already mentioned, a definite and recurring, though transitory, stage of the endogenous cycle in the vertebrate, and constitutes the negative period during which the short forms are not present in sufficient numbers, or are in an unsuitable state.

The very large number of cases where the attempted multiplication fails to establish an infection may be taken as representing the general inhibiting capacity of the *Glossina*, and appears to be a very fairly constant factor in all experiments dealing with newly-hatched flies. The great value from the experimental point of view of the infecting feed being the first feed ingested lies just in the point that only in this way can any sufficient uniformity be ensured in the condition of the alimentary tract of a batch of, for instance, 50 flies. The inhibiting capacity just mentioned is responsible, given a suitable condition in the infecting material, for keeping down the percentage of infected flies. Individual strains of *T. gambiense* vary greatly in the general percentage of infected flies produced, this being due to the greater or lesser vigour of the trypanosomes interacting with the inhibiting forces of the fly. The negative blood periods are just as marked in very vigorous strains as in those of lesser vitality.

(2) *Development of T. gambiense in the Gut of G. palpalis.*

To consider now the typical course of the developmental cycle of *T. gambiense* in *Glossina palpalis*. Some general features may be disposed of at once. There does not appear to be an intracellular stage at any part of the cycle, either in the gut or in the salivary glands. Such a development was very carefully searched for, Prof. MINCHIN and Dr. THOMSON's important discovery of this stage in *T. lewisi* naturally adding to the probability of an intracellular phase occurring in other cycles, though *T. lewisi* is obviously a very different type of trypanosome from any of those included in the "*brucei* group."

T. gambiense develops in the lumen of the gut from the very start and there is no gap or disappearance of the parasites from this situation at any period. The flagellates are never found to attach themselves in any way to the wall of the gut, but do attach themselves to the wall of the salivary gland when established there. In my experience the trypanosomes do not pass through the wall of the gut into the body cavity at any time.

While there is a very definite course of development in the fly before the trypanosomes are ready to infect another vertebrate, nevertheless the duration of this cycle of changes may vary to the extent of a full fortnight. A certain amount of experience with an individual strain will permit an observer to predict with considerable accuracy the state of affairs upon any given day; but it is quite impossible to say with a random strain that any type or condition is typical of a given date. A 10th or 12th day fly of one strain may correspond with a 20th day fly of another. Even a single strain may sometimes vary in regard to time as much as a week or ten days. This must be borne in mind in dealing with the stages of the cycle. Variation in the time-element of development is a well-known biological phenomenon, which has, however, not received much attention in dealing with Protozoa; the present instance is a very marked case. Flies may be infective already on the 17th day, while it is quite common for the cycle not to be complete until after the 30th day.

The earliest case obtained in which the trypanosomes could with certainty be considered as developing, and not merely persisting, is that of a fly of the 2nd day. That is to say, the trypanosomes had been in the gut 36 to 48 hours. A provedly developing infection of so early a date is merely obtained by a fortunate chance, and the evidence in favour of its authenticity is as follows:—Experiment 122 was fed on Monkey 597, an infected individual which was under close observation; both live and stained films were being examined daily. On the date of the infecting feed, March 5, 1912, 597 showed no trypanosomes in the course of the examination of the live film. Prolonged examination of a stained film, 3×1 in., showed very rare parasites. The fly in question (No. 8) died during the night of March 6-7 and was dissected at 9 a.m. on the morning of the 7th. It was found to contain partially digested blood and a very considerable number of trypanosomes, which were in an active and lively state, a number far in excess of those found on the stained film taken immediately before the cage was fed. It may be mentioned in passing that Experiment 122 afforded two more positive flies when the box was dissected on the 23rd day. Wet fixed films were made as usual from the contents of the gut of the fly and multiplying individuals were observed. Figs. 9-12 are from this fly. Although special attention was paid to this fly from Experiment 122, as being the earliest obtained, nevertheless the stages correspond very closely with those seen in the relatively large numbers of early positive flies dissected between the 3rd and 6th days. A reference to Figs. 9-16 will make this clear.

No very striking or rapid changes occur when the trypanosomes are taken into the gut of the fly. Degenerating forms are present

in greater or lesser numbers. The healthy-looking forms show a general but very slight difference from the blood type which is difficult to define in words; the membrane is slightly narrower; the kinetonucleus moves somewhat nearer to the trophonucleus, which itself in most specimens increases a little in size. There is no marked division into slender and broad forms; there is variation in respect to length and breadth, but the categories are not sharply marked off, and the extent of the variation is slight. One point, however, emerges, which is, I think, of real importance, namely, that the dividing individuals at this early stage are all relatively broad forms. Figs. 11 and 16 are typical of the process. A broadening of the individuals about to divide is not characteristic of the development in the gut of the fly as a whole, as a glance at Figs. 20 and 30-33 will show, the deduction being that in the earliest days it is the broad individuals that have been ingested that are capable of division. This observation tends to confirm the impression received from purely external experimental sources that the broader, shorter forms are responsible for carrying on the cycle in the fly.

The finer details of division will be gone into in the case of the later days, where the cytology of the process is exquisitely clear. One thing is, however, worthy of notice, and that is the relations of the kinetonucleus of the daughter individuals in Figs. 11 and 16. In both cases the young individual is still in a crithidial condition, a very transitory state, as it develops almost invariably into a trypanosome before it is set free. (See Fig. 12, from 2nd day.) This is only characteristic of the earlier divisions, and (with the exception of a very slender form to be discussed shortly) is the only sign of a crithidial phase to be observed in the gut development. There is, as will be seen hereafter, a most marked crithidial stage in this, as in most trypanosome cycles, but it does not occur in the gut. As multiplication proceeds there are produced a large variety of shapes and sizes, though the trypanosomes do not seem frequently to surpass the maximum length of the blood type, *i.e.*, 34-35 microns. By the 10th day or thereabouts there are very numerous trypanosomes exhibiting a wide range of form. The characteristic slender form so remarkable in the anterior intestine and proventriculus later in the cycle may begin to appear already, but only in small numbers.

A long thread-like form, such as that drawn in Fig 43, and which comes from a 12th-13th day fly (mid-gut), corresponds apparently to the "male" forms of Kleine and Taute.* There is no evidence in favour of this being a sexual form at all; it has never been found in conjugation, in spite of much searching, either by Kleine and Taute or myself. In dealing with flagellates, there is no support to be found, in any of the well worked-out cycles in which conjugation has undoubtedly been established, for the theory that markedly long slender forms are male gametes. In flagellates, all cases of conjugation involving hologametes (as must obviously be the case here) are practically isogamous.† There is

* 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' vol. 31, Part II.

† See DOFLEIN'S 'Lehrbuch der Protistenkunde' 1909, for a general account of the Flagellata.

no argument by analogy in favour of these long forms, such as Fig. 43, being males. The use of analogy is obvious in working with an individual of a very well-known group; no one would hesitate to use this method in working with the Coccidia, for instance, but the analogy, to be sound at all, must be drawn from the group in question.

In the case of *T. gambiense*, one must first establish clearly that these forms (Fig. 43) do enter into conjugation before they can be held to be male gametes. In the case in point, the evidence is against these forms being gametes. They have never been seen in conjugation with broad or other forms. They are of rare occurrence, and appear in the middle of a period of very active multiplication, which is not a characteristic of gametes generally. I have never seen them earlier than the 12th day, even in the most rapid cycles. Moreover, their nucleus and protoplasm has almost always a pathological appearance, and one is inclined to consider them as degenerating slender forms (compare Figs. 40-42).

A great deal of degeneration goes on in a well-infected fly, as is only to be expected when the tremendous numbers of parasites present are considered. These processes are easily recognised in the broader forms, but are much more obscure where the slender types are involved.

The posterior position of the nucleus sometimes seen in these forms seems to be due to the tendency so often shown in degenerating trypanosomes for all the protoplasm and its contents to aggregate at the posterior end (Fig. 41). This condition does not seem to me to be really in any way comparable to the usual crithidial phases of trypanosome cycles, where the whole culture passes through a state in which the trophonucleus is definitely posterior to the kinetonucleus, and which is followed by a re-assumption of the trypanosome-condition proper (compare the life-cycles of *T. lewisi*, *T. raia*, of the trypanosome of perch, goldfish, etc., the cycles of bird-trypanosomes, and the salivary gland phases of *T. gambiense*). Very slender individuals, without any trophonucleus at all, are seen at times; they are rare, and I do not consider that they are in any way normal forms. A limited vitality is preserved for a time in very degenerate specimens, and even in parts of a trypanosome, thus, actively motile flagella, with or without a portion of the membrane attached, are known to persist for some hours before disintegration sets in.

Cytology and Details of Division.

The cytological relations, and the details of division, are very well seen in the gut-forms, and the following account has been drawn from the middle period (10th to 12th day) of the cycle. The body-form, as already mentioned, is subject to the greatest variation. In the multiplicative types (Figs. 30-35) the protoplasm is granular and somewhat dense in appearance, but does not usually show larger inclusions. The kinetonucleus is larger than in the blood types, and the blepharoplast (or basal granule) of the flagellum is very clearly visible. The trophonucleus contains a large karyosome, apparently very rich in chromatin; the relations

are much as in the blood types, only the whole nucleus is generally rather larger, and the nuclear membrane is not quite so clearly marked.

At division, the blepharoplast divides before anything else, and is immediately followed by the first splitting of the flagellum. The two daughter blepharoplasts move to either side of the kinetonucleus, and very clearly and constantly play the part of centrosomes in its division (Fig. 30). A centrodesmose is formed between the two blepharoplasts, and division takes place without the formation of an equatorial plate. In well-stained iron-hæmatoxylin preparations, this set of conditions can be seen with the most exquisite clearness and precision. The earliest appearances of division in the trophonucleus are seen in Fig. 31, where the two centrosomes are lying on the slightly drawn-out membrane; they are joined by a centrodesmose, and the first signs of a division of the karyosome are to be observed. The nuclear membrane becomes very faint, but never seems to disappear, so that it seems highly doubtful in how far it is homologous to the nuclear membrane of the Metazoa. The chromatic material draws out along the lengthening centrodesmose into a somewhat irregular spindle, and the karyosomes ultimately re-form without the intervention of an equatorial plate phase. The remains of the spindle-figure, which is sometimes extraordinarily long (it may be much longer than that shown in Fig. 34), are absorbed in the protoplasm. The fate of the centrosomes after division is obscure, and I cannot say whether they are incorporated in the daughter-karyosomes, or whether they disappear or lie on the membrane. The division of the protoplasmic body is very characteristic. There is no longitudinal splitting of the parent-organism, but the young specimen is really as it were pushed off (or grows off) at the posterior end (Fig. 35), and the division is practically transverse. The two organisms never swing out so as to be arranged kinetonucleus to kinetonucleus, as in the majority of trypanosome-divisions. This behaviour is very constant; the young individual is usually somewhat smaller than the parent.

When the slender forms (Figs. 36, 37, 39) which constitute the regular proventriculus type come to be developed (this occurs any time after the 10th to 15th day) they arise quite gradually from the broader forms and there is only one point of importance in which they differ from their predecessors (Figs. 36 and 37). The body is long and slender, the protoplasm very finely granular and much less dense than in the broader forms, but the salient feature is the trophonucleus, which shows an interesting change. The karyosome has become very much smaller and the membrane has become much more marked and stains deeply, suggesting that it carries some quantity of chromatin; the fine rays can rarely be distinguished, but there are some indications that they nevertheless persist. There seems to be a reduction in the quantity of chromatic material in the whole nucleus, but it is impossible to say whether this is merely in relation to the lesser quantity of protoplasm, or whether it is some sort of a nuclear preparation for the subsequent development in the salivary glands. Division

takes place in fairly slender types (Fig. 38), but seems to be inhibited in individuals such as those shown in Figs. 36 and 37.

The infection as a whole generally develops in the hinder intestine or the posterior part of the middle intestine. I have never found trypanosomes established in the rectum. The infection literally grows forward by sheer force of multiplication till it fills the whole of the middle and hinder intestine and the posterior part of the anterior intestine. The anterior portion of the anterior intestine and the proventriculus show the typical long slender forms (Figs. 36 and 37) described in the preceding paragraph, and are not invaded till some time about the middle of the cycle, from the 10th to the 20th day, or thereabouts. There seems to be some difficulty in the trypanosomes reaching the proventriculus, and, once arrived there, they maintain themselves in that situation only so long as the fly is not exposed to too long a fast. If a fly has digested its meal and there is any considerable interval before the next is obtained, the trypanosomes ebb backwards to the posterior part of the anterior intestine, and only gradually recapture their position again. If, however, the new blood is taken in while the trypanosomes are still in the proventriculus, they retain their position and are apparently little affected by the influx of fresh blood.

These conditions can easily be observed from a series of simple experiments with boxes of the right age, in which the feedings have been suitably spaced and their relation to the state of the gut of the positive flies carefully observed.

This inability on the part of the parasites to maintain themselves in the proventriculus during a fast becomes of some importance in the cycle when the habits of the wild flies in regard to feeding, referred to earlier in this paper, are remembered. The majority of wild flies are obviously exposed to fasts of considerable duration, and it is this circumstance, combined with the peculiarity of the trypanosomes just mentioned, that seems to necessitate some seat of attachment for the trypanosomes anterior to the proventriculus in all cycles developing in *Glossina palpalis*. In *T. gambiense* the flagellates invade the salivary glands; in *T. pecorum* and *T. nanum*, as will be shown later, they attach themselves in the proboscis after having gone through a gut-development not unlike that of *T. gambiense*; in *T. vivax* and *T. uniforme* the whole cycle takes place in the proboscis. There is at present, as far as I am aware, no case of a completely investigated gut form carried by *Glossina palpalis* being transmitted directly from the alimentary canal without some definite secondary focus occurring anterior to the proventriculus.

In very numerous infections the trypanosomes sometimes overflow from the proventriculus into the crop, and may be found there in considerable numbers; they are typical slender proventricular forms. When injected into a clean monkey they are incapable of producing infection.

The gut-development may be said to culminate in the slender proventricular type. The hinder and middle regions of the intestine right up to the death of the fly present a medley of

multiplying forms differing little from those produced in the first 12 days. There does not appear to be much, if any, multiplication among the very slender individuals, and the hinder and middle intestine seems to serve as a reservoir from which the more slender types are being constantly drawn.

In many trypanosome cycles, notably those of fish and reptiles, this slender form is the last phase of the development in the invertebrate, but this is not the case in *T. gambiense*. An essential development differing very markedly from that carried on in the gut still remains to be gone through in the salivary glands. Before, however, leaving the gut cycle it is necessary to consider two very important points: (a) The meaning of certain curious multiple and non-flagellate forms found in the gut from about the 5th to the 12th day; and (b) the question as to whether conjugation has taken place at any period in the gut cycle just sketched.

(a) In live preparations of the gut there may occasionally be seen confused motile forms with several flagella; others are somewhat amœboid, and the flagella are more or less adherent to the protoplasm; others have no flagella at all, and are quiescent; and still others are obviously wriggling masses of half-fused trypanosomes. Figs. 21-24 give some of these forms as they appear in stained preparations. They always lie right up against the peritrophic membrane. Now, there is little doubt from the fact that these forms can be seen in live films to be caused by the fusion of soft, rather unhealthy-looking trypanosomes under the unfavourable condition obtaining on the slide, that many of these appearance are cases of degeneration. Nevertheless, this is no proof that all the multiple individuals are degeneration products, to be dismissed without further consideration. I have not been able to get any evidence that the multiple forms play any progressive or essential part whatsoever in the cycle. They must, however, always be borne in mind, as these appearances may afford an unfortunate mask, obscuring the process of conjugation. Sometimes in live films from the early days in the fly cycle, and occasionally also on films from the monkey when these were prepared with a little water or with a 0.50-per-cent. salt solution, trypanosomes were observed to come together from opposite sides in pairs so as to overlap about one-third of the body length as in Fig. 39A, which is from a freehand sketch of live individuals. The junction seems superficially to be extraordinarily close, suggesting fusion even under a high-power lens, nevertheless the specimens have always come apart again, the longest period of apposition observed being 35 minutes. Usually the individuals are quite similar to one another; on one occasion, however, in the case of a 3rd day fly, they showed a slight amount of differentiation, one being rather more slender than the other. The "male" type (Fig. 43), discussed earlier in the paper, was not involved in any of these cases, in all of which the trypanosomes were of the blood type.

This is the sum of the direct evidence from live observation that I have been able to obtain in the course of more than a year's search, and it is obviously inadequate. The trypanosomes do not

live long enough under the cover-slip to give satisfactory opportunities for this kind of observation. The fact that the divisions are so very characteristic and so constant in type would admit of valuable deductions being made from the stained material, were it not that the multiple degeneration and involution forms obviously rob these of all their value. It must also be considered whether conjugation might not take place in the salivary gland. Direct evidence there is none, and live observations are even more hopeless here, as the trypanosomes from the glands are extraordinarily sensitive to the unfavourable conditions of the slide. As will shortly be seen, there are important data for considering the salivary gland cycle as the really essential part of the whole development, and it is for that reason that one is inclined to entertain the idea that the sexual process might occur in this situation.

(b) Theoretically there is a good deal of evidence in favour of conjugation or some equivalent process occurring during the cycle in the transmitting host. The passage through the *Glossina* as a whole seems certainly to have the same biological significance. There is clear evidence in certain cases observed here at Mpumu—the case of Monkey 199 was cited in this connection in a earlier paper—that peculiarities acquired by a strain during its sojourn in a particular host are eliminated by passage through the fly, whereas these idiosyncrasies are retained when the strain is passed directly by injection. There can be no doubt that from a consideration of the life-history as a whole the part passed in the fly plays a definite and essential rôle in maintaining the integrity of the species, quite apart from its being a convenient method of transmission.

(3) *Stages of T. gambiense in the Salivary Glands.*

The penetration of the trypanosomes into the salivary glands occurs quite clearly from the hypopharynx, and the successive stages of the process can be seen very well in the live state, in careful dissections of the glands at the appropriate periods. An individual trypanosome cannot, of course, be watched through the process, but the study of a good number of flies leaves no shadow of doubt as to the sequence of development. The easiest way to get at the thin duct of the glands, which is the place where the first stages are to be found, is to starve the fly for full two days, then to cut off the posterior tip of the abdomen, snipping off a slightly larger portion than is usual for ordinary dissection, and to pull out the gut without breaking it off. This minimises the chance contamination of the gland with the contents of the gut. If the glands do not appear, stroke the abdomen gently with the flat of the needle until they do; when the tip of the gland emerges, a single rather slight stroke of the needle will bring it out right up to the narrow duct, and the same for the other side. The glands are transferred to a clean slide with a drop of normal salt solution or monkey serum; they are transferred again to a clean slide and examined. The pulling out without breaking the gland is an

easily acquired knack and should be done under a low-power dissecting microscope.

The slender trypanosomes pass up into the hypopharynx from the proventriculus. The period at which this happens is in direct correlation with the vigour and number of the trypanosomes in the fly, and early infectivity is generally a character found in a strain which produces many positive flies. These slender trypanosomes may in rare cases be seen lying, in small numbers, free in the hypopharynx of flies whose salivary glands are not yet infected. They then come back along the narrow duct of the salivary glands, and can be seen there as free-swimming slender creatures.

The gland, as is well known, is composed of (1) a narrow tubular part, which leads back to (2) a slightly broader cellular part, which in turn leads to (3) the glandular part, where the full width of the organ is attained.

The trypanosomes settle down and attach themselves in the second part of the gland, or at the entrance to the third part, the rest of the gland being quite free from trypanosomes. At first they are slender forms, which sway forwards and backwards, attached by their flagellum to the wall of the gland (Figs. 44 and 45). Later on they become broad, round-ended, crithidial flagellates. They multiply, and gradually, what with divisions and fresh arrivals, finally fill up large portions of the gland with flagellates in all stages, from tadpole-shaped crithidias, attached by their flagellum, to free-swimming trypanosomes, closely resembling the blood type (Figs. 44-60). These trypanosomes are usually below the length, but not below the minimum measurement, of the forms occurring in the blood. Division takes place among the trypaniform, as well as among the crithidial, parasites (Figs. 49, 56, and 57). The cytology of the gland forms calls for no special notice, except that there seems to be an increase of chromatin within the nucleus, and there is a tendency for the karyosome to increase once more in size. The occurrence of marked crithidial, and in some cases almost herpetomonad, forms is very striking; they constitute a large proportion of all the gland types. Degenerating individuals are found in all stained films, but the great sensitiveness of the trypanosomes to the process of investigation leads one to imagine that some of these at least are due to manipulation. While the crithidial forms are mostly attached, they may, nevertheless, be found free also; it seems probable, however, that they attach themselves again.

The fly is, apparently, already capable of producing an infection in the vertebrate when only the proximal part of the gland close to the duct shows parasites. The *Glossina* seems to become infective about 2 to 5 days after the trypanosomes invade the gland, but, as one cannot both dissect and subsequently observe an individual fly, this time is naturally only an estimate made from the consideration of a number of only more or less similar cases. It appears to be clear, beyond question, that only when the salivary gland shows trypanosomes is the fly infective. Early positive transmissions are always accompanied by rapid virulent infections in the fly and an early invasion of the glands. In one

case a salivary gland was found infected on the 12th day, but this is quite exceptional—on the 16th day infected glands are found in rare cases, and after the 20th day they become increasingly frequent.

CYCLES OF *T. nanum* AND *T. pecorum* IN *G. palpalis*.

It is interesting briefly to compare the general course of development in *T. nanum* and *T. pecorum* with that of *T. gambiense*. The development of *T. nanum* in *Glossina palpalis* has many features that closely resemble the conditions in *T. gambiense*. The infection starts in the hinder intestine, and by the 10th day (no material was obtained from the very earliest days) numerous trypanosomes are to be found in the hinder and middle intestine. They show a considerable range of form, and have nuclear relations practically identical with those of *T. gambiense* (Figs. 61-64). The method of division corresponds in such minute detail with that described in Figs. 30-35 for *T. gambiense* that it is needless to recapitulate the description. Slender forms begin to be produced from the 10th to the 14th day of the cycle onwards, and the proventriculus comes to be invaded about the 20th day. The proventricular forms, while generally slender, do not show such a uniformity of type, nor are they so thread-like, as in the case of *T. gambiense*. Moreover, the characteristic change in the nucleus of *T. gambiense* shown in Figs. 36 and 37 never takes place at all in the gut forms of *T. nanum*. About the 25th day trypanosomes begin to be found in the proboscis attached to the labrum, often lying in clusters. They assume the crithidial condition, many of them being extraordinarily long and slender (Figs. 65-68). No reliable information was obtained concerning the nuclear detail of these types (the drawings are all from dried Giemsa preparations), as it was found that the trypanosomes could not be made to adhere to the cover-slip in the wet preparations, except in very rare instances. Besides the very long crithidial types, shorter forms, such as that shown in Fig. 68, were observed, also crithidial in type. In live preparations, free trypaniform organisms were observed, sometimes in the hypopharynx and sometimes in the labrum.

As in *T. gambiense*, the gut forms do not attach themselves to the wall of the alimentary canal, nor are crithidial forms ever seen in the gut cycle. The salivary glands are never invaded in the case of *T. nanum*, the proboscis-infection apparently playing the rôle of the gland phases in the cycle of *T. gambiense*.

Trypanosoma pecorum develops in *G. palpalis*, but the cycle is so extraordinarily slow, and the transmission so difficult to effect, that it appears clear from Dr. H. L. DUKE's experiments that this fly is at most only a facultative intermediate host for *T. pecorum*. For this very reason, the conditions of the cycle have some points of special interest, as will be seen later. The course of the development in the gut resembles that of *T. nanum* and *T. gambiense*; multiplication occurs, and numerous types, varying in length and breadth, are formed (Figs. 69-71). The flagellates are more massive and larger than in either of the two

forms just mentioned. The slender forms are produced as usual after the broad individuals, and are found in association with them; the long forms are extraordinarily attenuated, and the nuclear change remarked upon in the corresponding stages in *T. gambiense* occurs also here (Fig. 72). The first invasion of the proventriculus was observed upon the 45th day of the cycle, and no proboscis-infection was found before the 76th day. The proboscis-infections were generally slight, and the usual difficulties were experienced in getting preparations from this situation. Figs. 73 and 74 show two individuals from a wet preparation, fixed in Schaudinn's fluid, and the nuclear relations recall those of the gland-stages of *T. gambiense*. The salivary glands never become infected.

GENERAL CONSIDERATIONS AND CONCLUSIONS.

The most remarkable feature of the cycle of *T. gambiense* is the curious double development, first in the gut, culminating in the long slender form, and then in the salivary glands, where the crithidial stage so typical of all trypanosome cycles is so marked a feature. It seems difficult to escape the deduction that the gut-development is a somewhat indifferent multiplication, a mechanical device to enable the trypanosomes to establish themselves in sufficient numbers in contact with the salivary fluid, which in the fly seems alone capable of stimulating the trypanosomes to that apparently essential reversion to the crithidial type. In *T. pecorum* and *T. nanum* the gut-development follows in all essentials the scheme of *T. gambiense*, and here also, as already mentioned, the proventricular forms are not infective. While the double nature of the cycle is perhaps more obvious in *T. gambiense* than in *T. pecorum* or *T. nanum*, the cases are, nevertheless, close parallels. The parasitism in the case of *T. gambiense* is obviously of a more complete type, as the invasion of the salivary glands lends greater permanence and stability to the apparatus of infection than the more or less intermittent infection of the proboscis found in *T. pecorum* and *T. nanum*.

In accordance with the foregoing, it may be said that the gut-conditions of *G. palpalis* do not permit of the complete and essential development of any of the group of trypanosomes mentioned in this paper. So long as the parasites are established in the gut only, their presence is indifferent and negligible from the point of view of infection. This is very striking in those rather rare cases where flies infected with *T. gambiense* may show considerable numbers of flagellates as late as the 56th day without the salivary glands being infected. Such flies are invariably harmless. *T. pecorum* may also be considered in this connection; the culture in the gut persists for 70 days in a harmless state, only becoming infective when the parasites do at length succeed in establishing themselves in the proboscis, and going through the crithidial development.

In conclusion, it may be remarked that, if a sexual process is a necessary feature of the cycle passed in the transmitting host, it will probably occur at that stage which seems absolutely essential

to the production of a trypanosome viable in the blood of the vertebrate, namely, the crithidial phase in the salivary glands or proboscis.

It is obvious that much of the foregoing work has been simply to carry somewhat further the researches of Minchin, Roubaud, Bruce, and Kleine, more especially of the last two workers. There are no serious discrepancies between the cycle in the fly sketched by Bruce, Hamerton, and Bateman, and that described above, except that I consider, as has already been said, the fly-history to be in reality a double development. In many points my work is also in agreement with that of Kleine and Taute, except that I do not consider that the "male" forms described by him play any important part in the cycle.

A further discrepancy consists in the view held by the German workers at the time of writing their paper, in regard to the salivary gland phases being a non-essential part of the cycle. My interpretation of the endogenous cycle in the blood of the vertebrate is at present, as far as I am aware, unconfirmed by other workers, largely, I imagine, owing to the fact that the interest has been concentrated for some time past on the appearances in the fly rather than on those in the vertebrate.

Summary.

T. gambiense undergoes an endogenous cycle of development in the vertebrate in the circulating blood. This cycle is of irregular duration and is repeated many times in the course of the disease.

2. The short forms may be regarded as the adult blood-types; the intermediate types are growth-forms, proceeding to the long individuals, which are those about to divide. The products of division give rise, directly or indirectly, to the adult forms. The adult forms appear to be alone responsible for carrying on the cycle in the transmitting host.

3. The multiplication occurs in the circulating blood.

4. Multiplication of the parasites was never found within the cells of the liver, spleen, or lungs in monkeys.

5. Rounded non-flagellate types were found on one occasion in the lung, liver, and spleen of a virulently infected monkey. They appear for the most part to be destined to destruction, but it is not excluded that they may survive in small numbers as latent forms.

6. In the fly the trypanosomes are first established in the posterior part of the mid-gut. Multiplication occurs and trypanosomes of very varying sizes are produced.

7. From the 10th or 12th day onwards slender long trypanosomes are to be found in increasing numbers. These finally move forward to the proventriculus and are the dominant, though not the only, type seen there. The proventriculus becomes infected as a rule between the 12th and 20th days.

8. The salivary glands become infected by the slender proventricular types. They reach the salivary glands by way of the hypopharynx; arrived in the gland, they become attached to the wall and assume the crithidial condition. Multiplication occurs and finally small trypanosomes are produced, closely resembling

the blood type. The passage through the crithidial stage is the characteristic of the salivary development and the trypanosome forms just mentioned are derived from the crithidial types. The development in the salivary gland takes from two to five days before the forms are infective.

9. The fly is never infective until the glands are invaded. Trypanosomes from the proventriculus when injected into a monkey never produce infection. Trypanosomes may be found in the salivary glands as early as the 16th day of the cycle. An early infection of the salivary glands is always preceded by a very virulent and rapid gut infection.

10. The trypanosomes are never attached to the wall of the alimentary canal, and there is no intracellular multiplication in the gut cycle. A crithidial stage does not occur in the gut cycle. The trypanosomes are never found in the body cavity nor are they ever established in the rectum.

11. Conjugation has not been observed, nevertheless the fly cycle as a whole has the biological significance of conjugation.

12. The cycles of *T. nanum* and *T. pecorum* agree with that of *T. gambiense* in showing a gut development without a crithidial phase. The crithidial phase occurs in the proboscis, where the flagellates attach themselves. The salivary glands are never infected in the case of *T. nanum* and *T. pecorum*.

DESCRIPTION OF PLATES 2-7.

The figures are all drawn at an approximate magnification of 3,000 diameters, with the aid of the drawing apparatus of Abbé.

Trypanosoma gambiense.

- Figs. 1-4.—Trypanosomes from blood of monkey.
 „ 5-8.—Division of blood types.
 „ 9-10.—Trypanosomes in the middle intestine of *Glossina*, 36-48 hours after ingestion.
 „ 11-12.—Division in the middle intestine, 36-48 hours after ingestion.
 „ 13-15.—Forms from the hinder intestine, 3rd to 4th day of cycle.
 „ 16.—Division in the hinder intestine, 3rd to 4th day.
 „ 17-19.—Forms from the middle intestine, 5th day.
 „ 20.—Division from 5th day.
 „ 21-23.—Multiple forms from the 6th day of cycle; 21 is obviously a degenerative appearance.
 „ 24.—Involution form from 6th day.
 „ 25-29.—Miscellaneous gut forms from the 12th to 20th day.
 „ 30-35.—Details of division.
 „ 36-37.—Slender proventricular types, final form of the gut development.
 „ 38-39.—Slender forms in middle intestine.
 „ 39A.—Sketch of live trypanosomes, from slide, from the middle intestine on the 3rd day.
 „ 39B.—Non-flagellate form from liver smear of Monkey 653,

- Figs. 40-45.—Early slender forms degenerating in the middle intestine; 43 seems to correspond to the "male" type of Kleine.
 „ 44-45.—Specimens newly arrived in the salivary gland.
 „ 46-55.—Typical salivary gland forms; note the crithidial condition.
 „ 56-57.—Division figures in the salivary gland.
 „ 58-60.—Final trypanosome types in the salivary glands, probably the infecting form.

T. nanum.

- Figs. 61-63.—Gut types from 14th day.
 „ 64.—From the proventriculus, 21st to 25th day.
 „ 65-68.—From the proboscis, crithidial forms; note length of 65, stained by Giemsa, dry method.

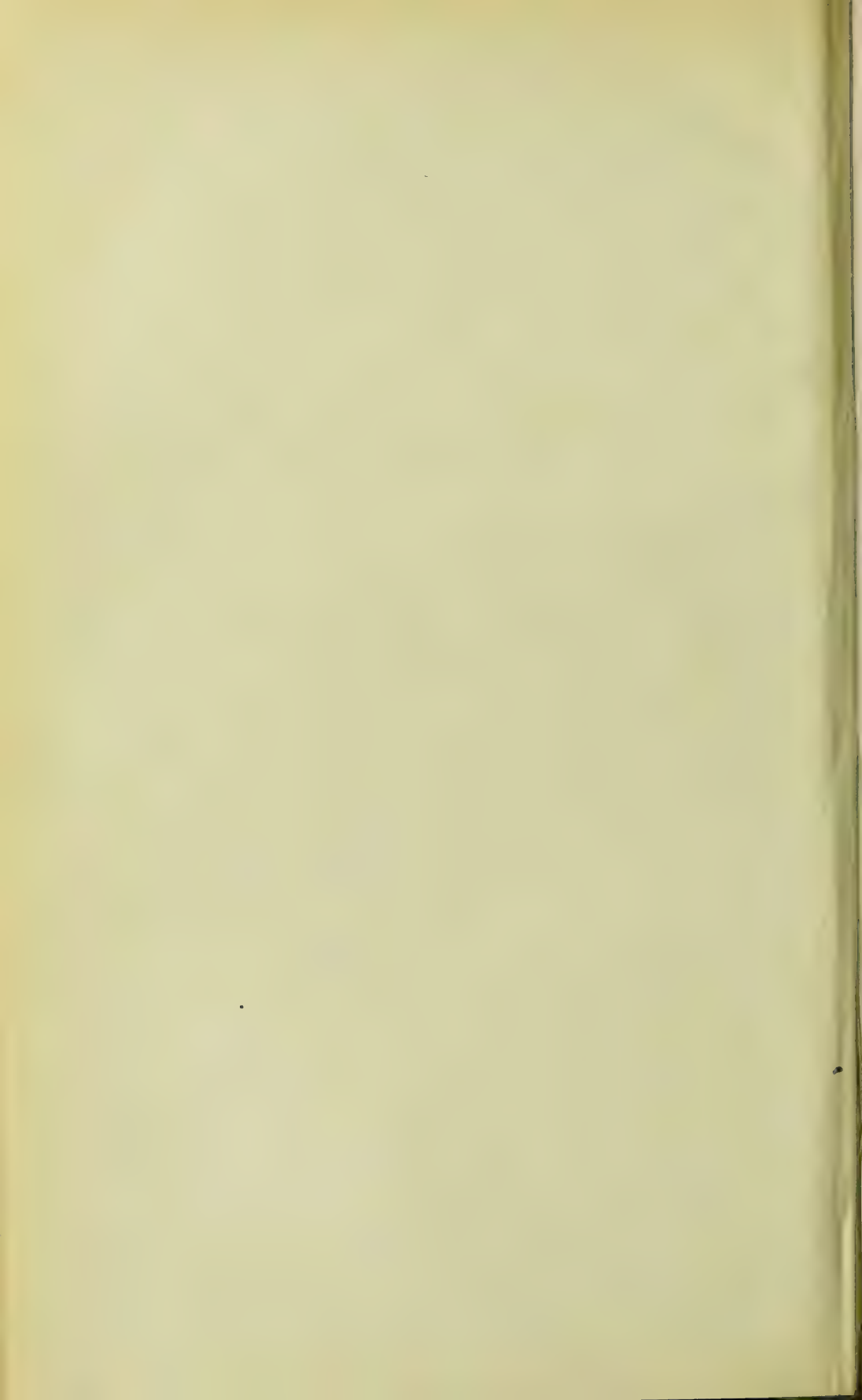
T. pecorum.

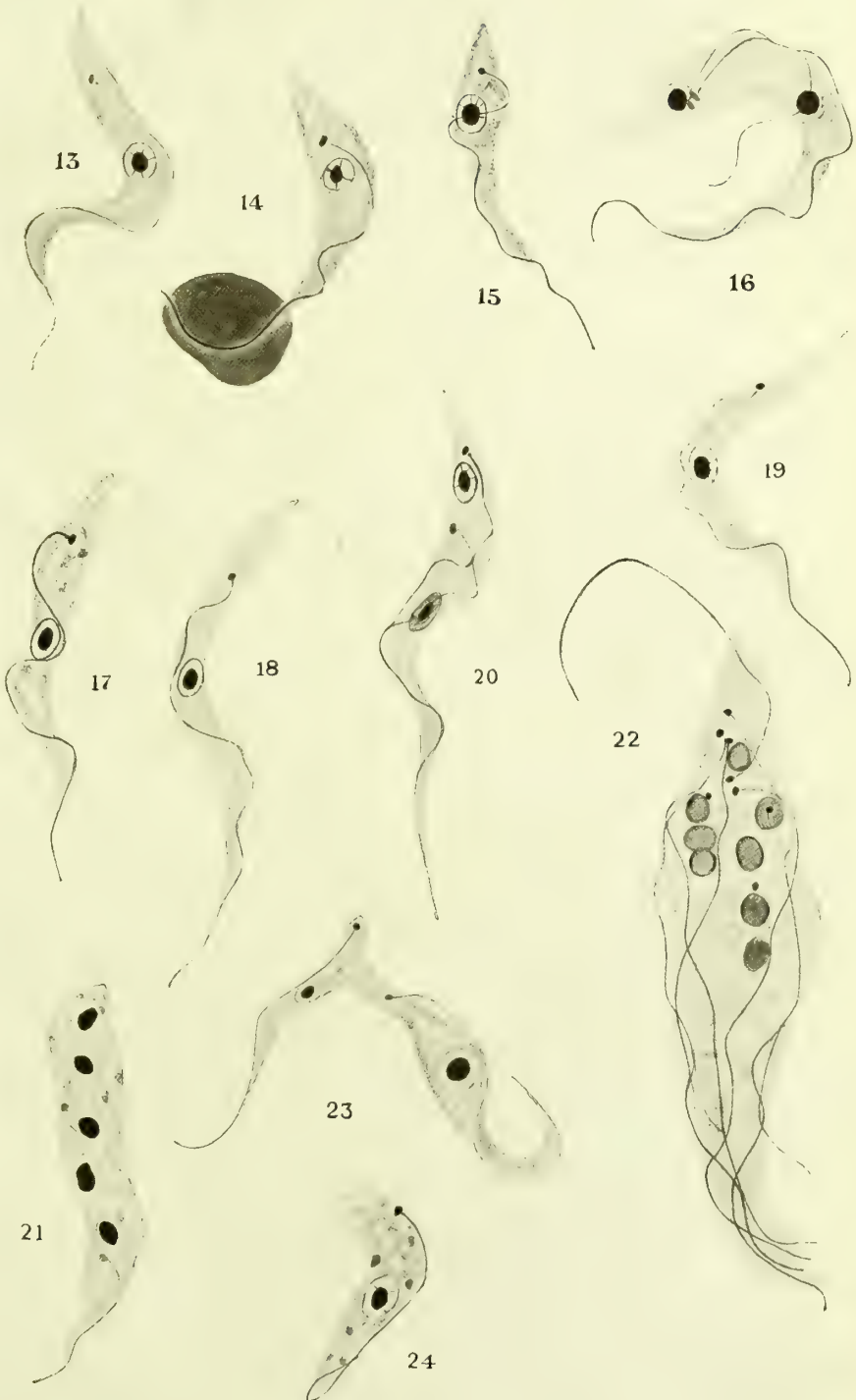
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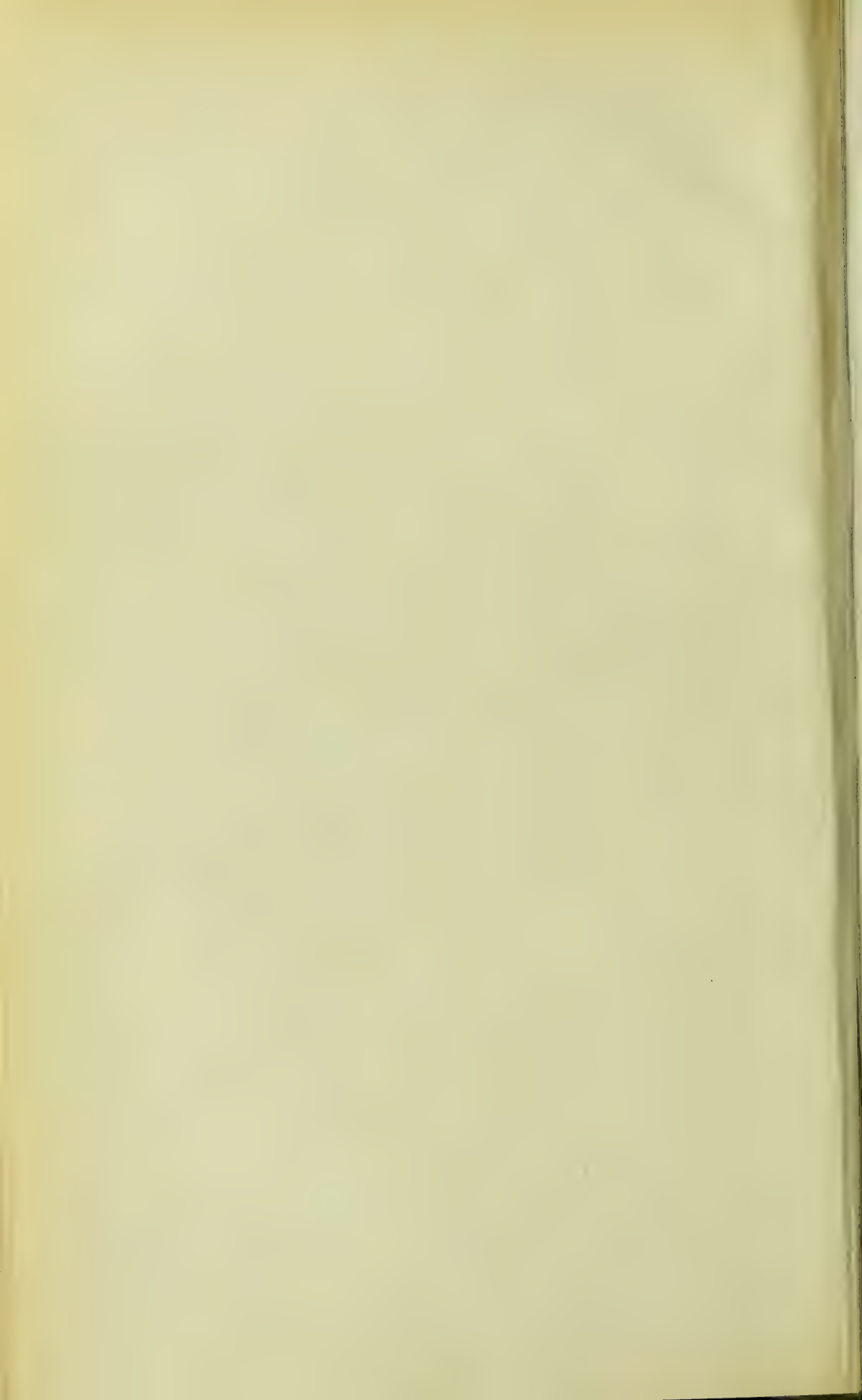
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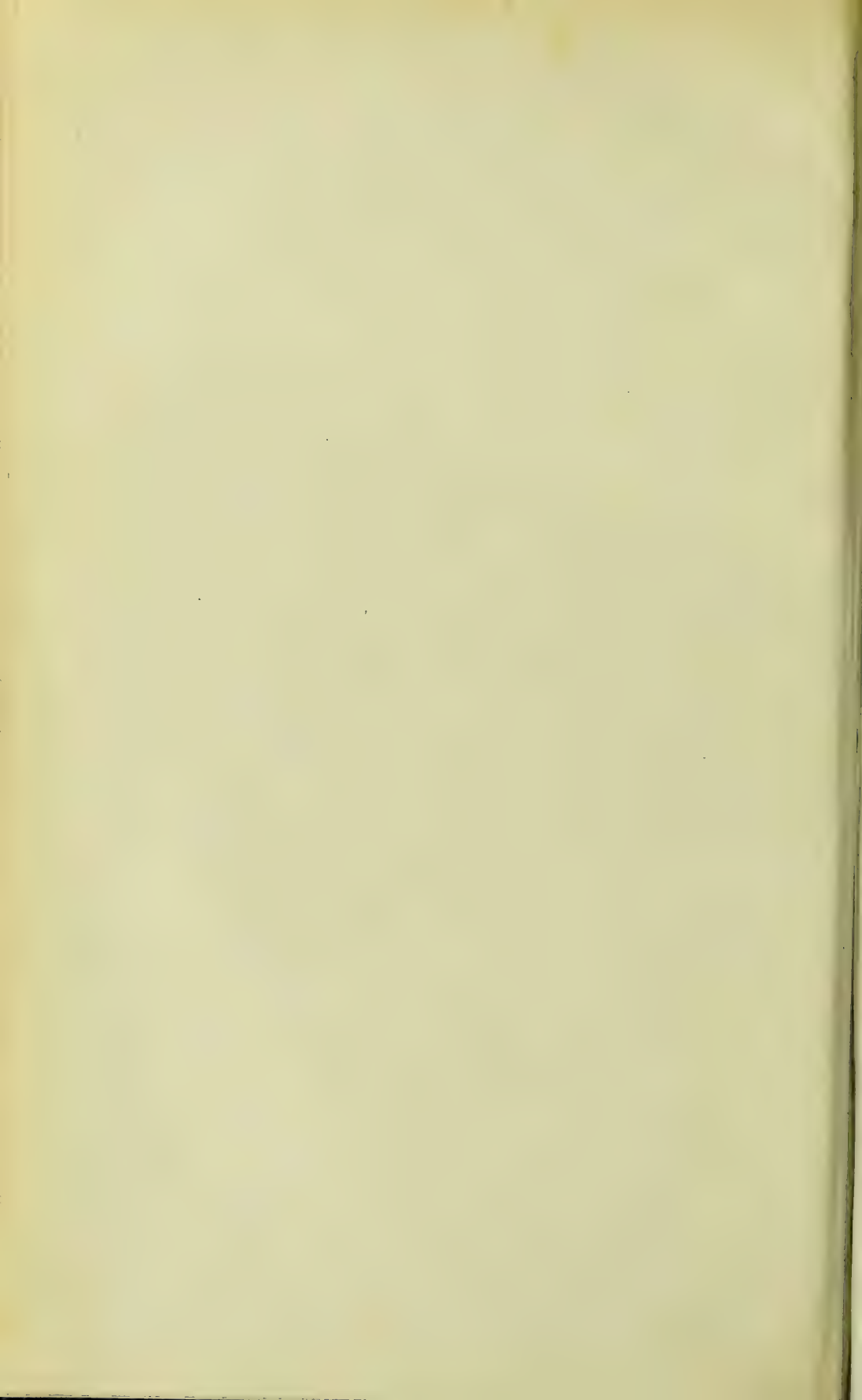




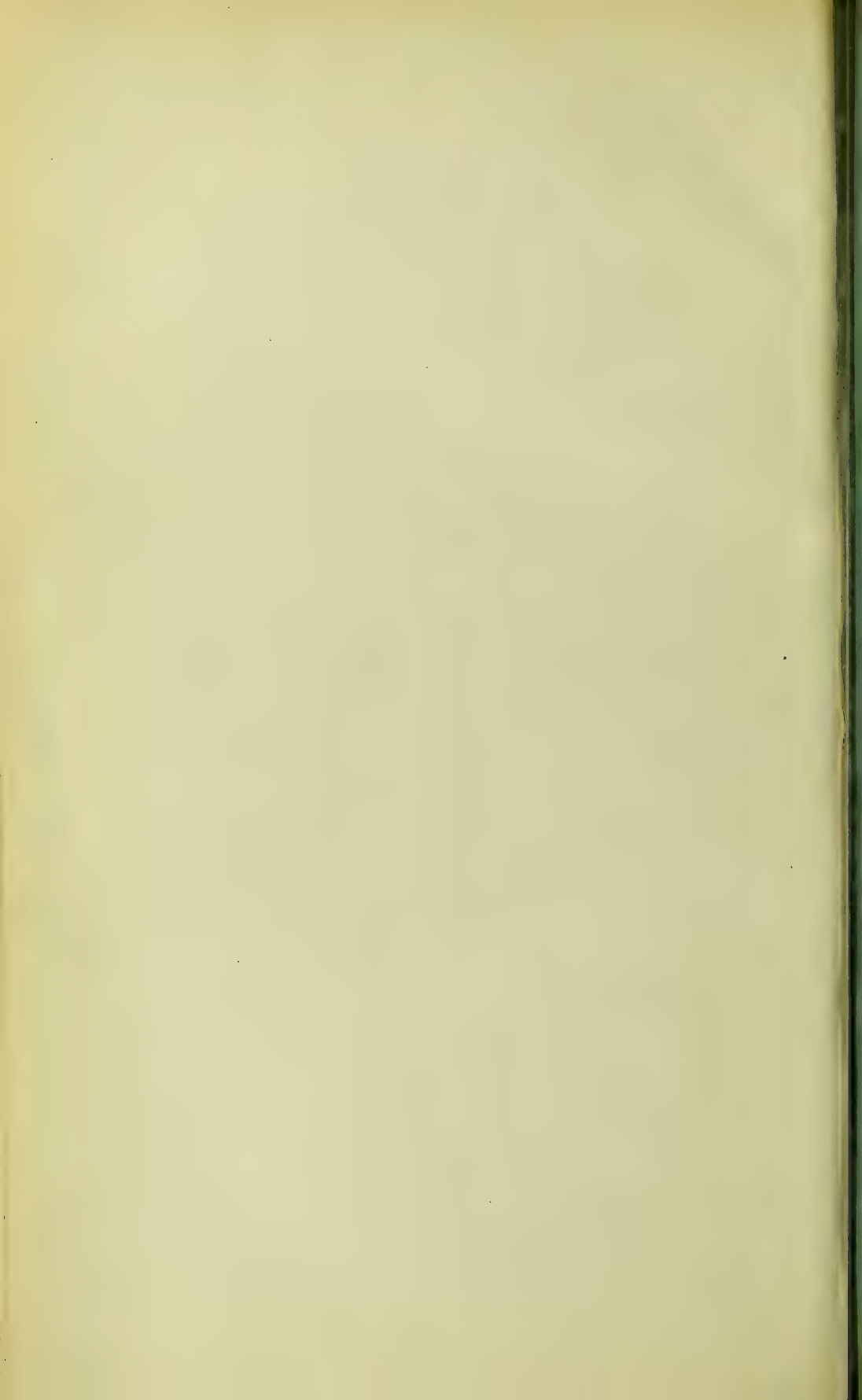






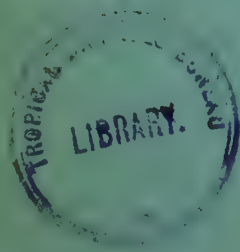




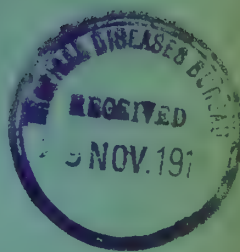


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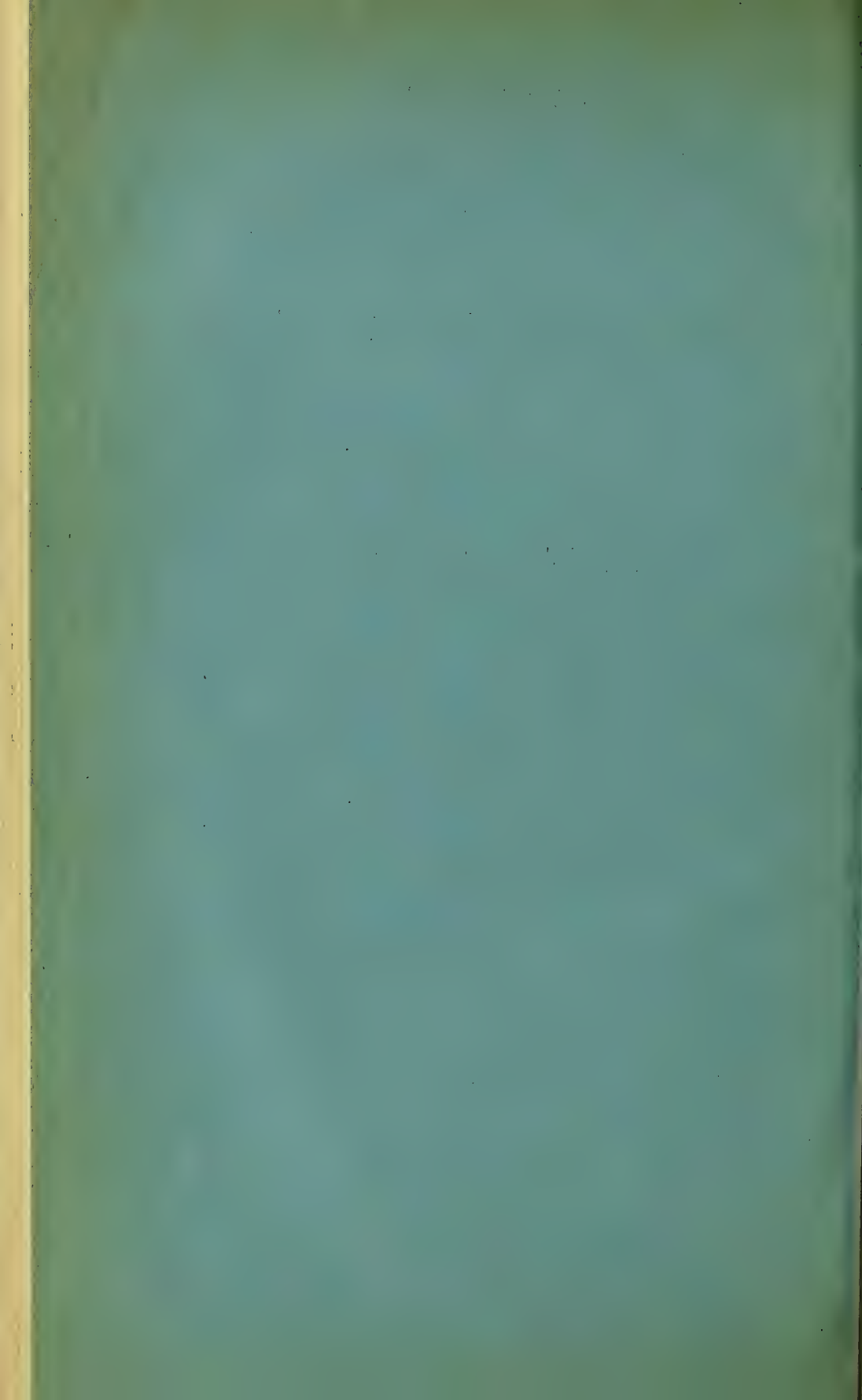
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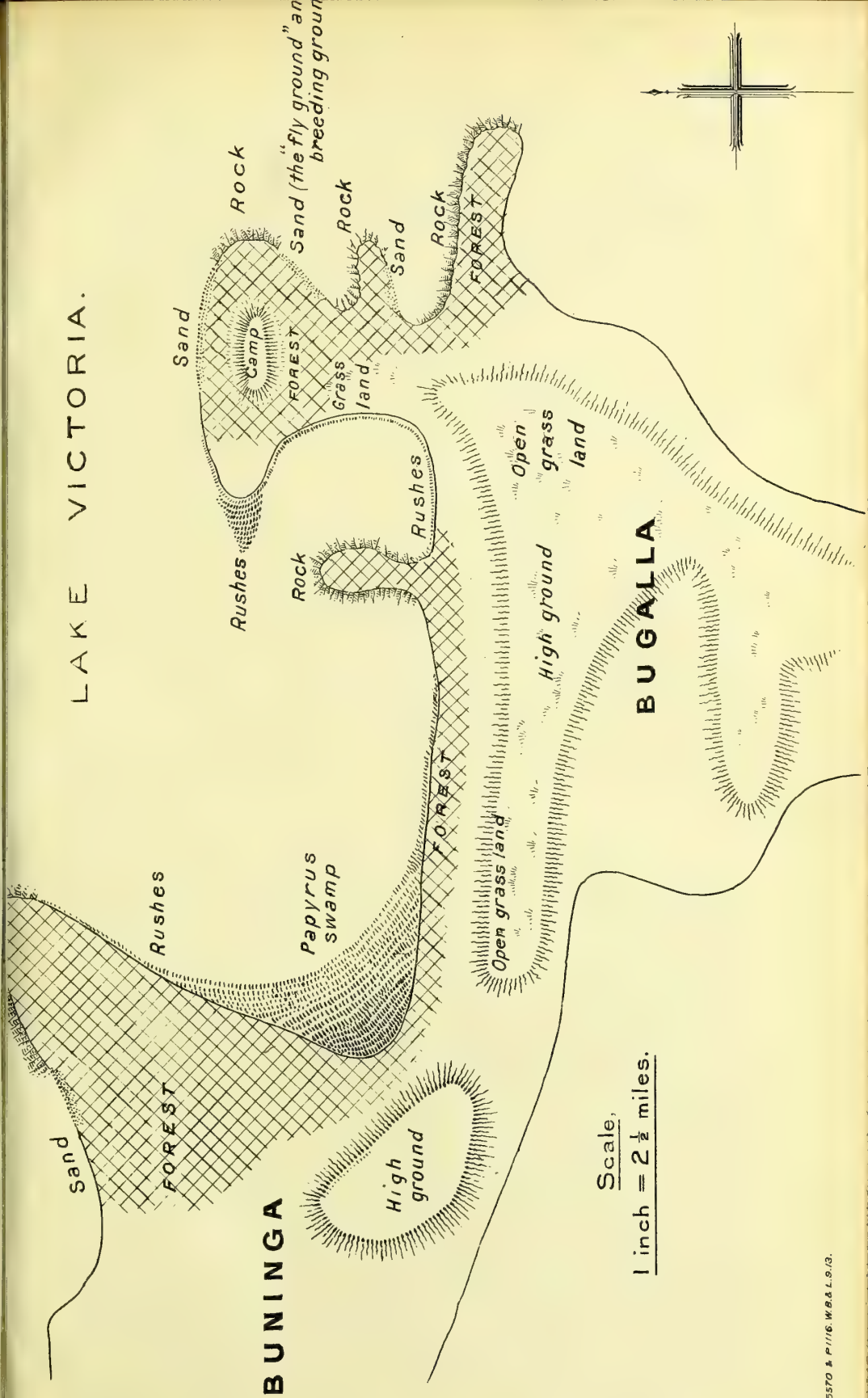
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LAKE VICTORIA.



Scale,
1 inch = 2 1/2 miles.



SECOND REPORT ON THE BIONOMICS OF GLOSSINA FUSCIPES (PALPALIS) OF UGANDA.

G. D. HALE CARPENTER, B.A., D.M. (Oxon), F.E.S.
(Uganda Medical Service.)

PRELIMINARY.

The Progress Report sent from Damba Island in September, 1911, and printed in Volume XII of the Sleeping Sickness Reports contained an account of the work done up till August 5th of that year.

From then until the end of the year the work was continued on Damba Island. I then, after a fortnight at Entebbe, moved to the Sesse Islands in the north-west corner of Lake Victoria, and after preliminary investigations settled in the north-east corner of the biggest island, Bugalla, near the site of the former Sleeping Sickness camps, mission, &c. The preliminary looking round, and the necessary house building, etc., made it impossible to settle into routine work until the commencement of March, 1912; twelve months were then spent in continuous work at this spot.

The nature of the locality is indicated diagrammatically on the accompanying map.

DESCRIPTION OF LOCALITY.

The camp was situated on a small peninsula, on the central hill of which, grass clad and about 200 feet above the lake, the grass hut was built.

The foot of the hill was encircled by a narrow belt of forest, only 100 to 200 yards wide, fringing the coast, save on the western side, where, owing to the subsidence of the lake level, a strip of open grass land lay between the edge of the forest and the water.

Here the coastline was formed by rushes which stretched out into the lake, making a promontory.

The northern coastline was either rocky or sandy, the latter forming a considerable stretch of foreshore backed by forest. Where the coastline was *rocky* the forest came to the water, where *sandy* there was a bit of foreshore. As will be seen later, however, this was not a suitable locality for finding large numbers of pupæ.

On the eastern side of the peninsula was a bay, part of the coastline of which was a sandy foreshore, the rest being rocky. This piece of foreshore was made the "fly ground," for flies were more numerous there than elsewhere, and also pupæ were

to be found collected there, which was not the case on the northern sandy strip. The accompanying photograph shows this fly ground. (See Fig. 1.)

South of this bay was another, with a smaller stretch of sandy foreshore, where only a few dozen pupæ could be found at one time, the shade being insufficient.

The coastline of the western side of the peninsula, fringed with rushes, without shade, and fly free, swept round a bay to a small, forested peninsula with rocky shore, and then was continued west and north, forming a huge bay.

The whole southern shore of this was fringed with rushes—which partly filled up the angle of the bay—and backed by forest which covers the whole of that flat part of the island known as Buninga.

The coastline here was rocky only, but round the point where it turned westwards again a little sandy stretch was found, only some twenty yards long, where a few pupæ could be found, but only a small number, the shade being very limited.

It is seen, then, that along many miles of coastline there were only four localities where collections of pupæ might be expected, and of these only one, on investigation, yielded any number of pupæ. The subject will be returned to under the heading Breeding Grounds.

The centre of the island is formed of open grassy downs, rising some 200 to 350 feet above the lake, with small patches of forest where there are little rivulets during months of greatest rain.

THE GLOSSINA.

The species of *Glossina* which is the subject of this and the previous report, has been shown by Dr. MacConnell, of the Uganda Medical Service (*Bull. Ent. Research*, Vol. 3, Part I., May 1912, p. 59) to correspond fairly well with that which was described by Newstead (*Bull. Ent. Research*, Vol. 2, Part I., May 1911, pp. 26-27) as *G. fuscipes*, from a specimen sent from the Nile at Nimule.

I have examined the hypopygia of five dozen of the Sesse Islands flies and compared the structures with those of flies from the mainland (for which I am indebted to Dr. H. L. Duke) and from Damba Island.

They all agreed exactly, so that this and the former report deal only with the form described as *Glossina fuscipes*, and not the true *G. palpalis*.

It was also thought that it would be of some interest to examine hypopygia of flies from a batch which had been fed upon an infected animal, only some of which had become infected with trypanosomes, in order to see whether the fact that only a small percentage becomes so infected may be accounted for by the fact that such are variations from the type, and whether the variation might show itself in the hypopygial structures.



FIG. 1.
Fly Ground.



FIG. 2.
A haunt of Situtunga.



FIG. 3.

Genital Armature of the Glossina.

I am indebted to Dr. H. L. Duke and Miss Robertson, of Mpumu, for sending me hypopygia from a batch of flies, some of which had become infected and others not. Preparations made from these former, however, showed no appreciable difference from any others that had been examined, whether from mainland or islands.

Fig. 3 shows the essential characteristics of the hypopygial structures of the *Glossina* of Bugalla Island, Sesse, and of Uganda generally. On careful analysis, however, the structures show some of the characteristics described as typical of *G. palpalis* and some of those of *G. fuscipes*.

Superior claspers.—These are connected by a deeply divided membrane, beset with short spines, as is the case with all members of the *palpalis* group. They have the "broad, beak-shaped base" described for *palpalis* (Newstead, *loc. cit.*), but distally they are not "curved suddenly inwards on the outer margin," as is described for the typical *fuscipes*.

The tip is "suddenly attenuated and tooth-like," which is a character of *palpalis*, and not "claw-like," as in *fuscipes*. The base is strengthened by ridges of chitin between which it is very thin. The bristles along the outer margin are long and fine, and there are usually three near the base of the tooth at the tip of the clasper, but sometimes only one, as in *palpalis*. Along the inner margin the bristles are shorter and more spinose and decrease in length from base to apex; they rise from thin areas of chitin, as in the case in *fuscipes*.

The inferior claspers correspond in every way with those of *fuscipes*, and are markedly different from those of *palpalis*.

The juxta and harpes, on the other hand, seem to be in accordance with the characters given for *palpalis*.

The *editum* is of the type common to both.

LENGTH OF LIFE OF THE FLY.

The previous experiments to determine the longevity of the wild fly, carried out at Jinja (see *Progress Report*, Vol. XII., of *Reports of Sleeping Sickness Commission*) were performed after the heaviest rains of the year, and not long before the driest months.

It was thought advisable to repeat the experiment immediately on arrival at Bugalla so that the flies would be marked at the most favourable time of the year and would have the opportunity of living for many months, before the driest weather came, the Jinja experiments having rendered it probable that this is inimical to the flies.

The markings were carried out as at Jinja, viz., by amputation through tibia or femur of one of the legs. The whole of the flies caught each morning were put into a cage and taken out one by one and marked at the close of several hours catching, when the number of those previously marked was noted.

For the period of about a week all flies caught, which had not been previously marked, were marked in one manner; the next week's catch was marked in another manner, and so on. The possible markings were twelve, numbered in rotation round the body, commencing with No. I. through the right fore tibia, No. II. through right fore femur, No. III. through right mid femur, and so on.

Altogether, five markings were used, and a total of 9,012 flies were so marked between March 18 and April 26, 1912, as seen in Table I. below.

LONGEVITY OF FLY.

Table I.

Date.	Mark.	♂	♀	Total	Mark 2.	Mark 3.	Mark 4.	Mark 5.	Mark 6.
					♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀
March 18	2	235	74	309					
" 19	"	346	92	438	3				
" 20	"	232	47	279	1				
" 21	"	268	61	329	10 1				
" 22	"	275	59	334	11				
" 23	"	236	66	302	19 3				
Total ...		1,592	399	1,991					
March 25	3	221	53	274	11 1				
" 26	"	348	81	429	13	15 2			
" 27	"	335	90	425	11 1	21 4			
" 28	"	303	72	375	7	20			
" 29	"	219	62	281	19 1	20			
" 30	"	235	80	315	16 1	12 4			
Total ...		1,661	438	2,099					
April 1	4	236	57	293	18	28			
" 3	"	276	73	349	14	15 1	4		
" 4	"	198	38	236	9	14	4		
" 5	"	110	15	125	5	4 1	6		
" 6	"	217	40	257	7	19 1	10 1		
Total ...		1,037	223	1,260					
Apr 1 8	5	293	90	383	9	8	5		
" 10	"	342	88	430	4	12 1	9	4	
" 12	"	174	42	216	3	7	5	2 1	
" 13	"	373	79	452	5	13 2	16	26 2	
Total ...		1,182	299	1,481					
April 15	6	269	73	342	7	9	10 1	15	
" 17	"	254	105	359	4 1	6	8	18 1	4
" 22	"	280	99	379	3	7	5 1	14 2	11 2
" 24	"	363	72	435		8 1	7	19 1	5 1
" 25	"	353	76	429	3	6	7	28 1	11
" 26	"	174	63	237	2	2	4	9	16 1
Total ...		1,693	488	2,181					

Date.	Unmarked flies caught.			Marked flies re-caught.				
	♂	♀	Total.	♂ 2 ♀	♂ 3 ♀	♂ 4 ♀	♂ 5 ♀	♂ 6 ♀
April 29 ...	310	69	379	1	3 1	4	6	16
" 30 ...	355	89	444	1	3 2	3	7 2	22 1
May 2 ...	338	85	423	3		3	6 1	19 1
" 3 ...	262	109	371	3		3	6 1	20 1
" 6 ...	298	100	398	1		1	2	8
" 8 ...	359	58	417			3	3 1	8 1
" 11 ...	281	68	349				1	6 1
" 15 ...	554	160	714		1	1	2	10 2
" 18 ...	339	111	450		2		3	3
" 20 ...	328	85	413				1	2
" 22 ...	138	25	163		1			1
" 23 ...	144	59	203				1	
" 28 ...	305	44	349		1		1	1
" 30 ...	238	59	297				2	
" 31 ...	256	62	318					
June 1 ...	229	49	278					
" 3 ...	169	33	202					
" 5 ...	184	48	232				1	1
" 11 ...	247	25	272					
" 12 ...	252	45	297					
" 13 ...	177	42	219			1	1	
" 14 ...	161	26	187					
" 17 ...	142	33	175					
" 19 ...	95	16	111					
" 20 ...	318	99	417					
" 26 ...	57	40	97					
" 27 ...	113	107	220		1			
" 28 ...	102	52	154					
" 29 ...	355	143	498					
July 3 ...	265	87	352					
" 6 ...	333	90	423					
" 9 ...	236	90	326					
" 11 ...	323	131	454					
" 12 ...	157	34	191				1	
" 17 ...	180	20	200					
" 18 ...	259	78	337					
" 19 ...	300	99	399					
" 20 ...	237	91	328					
" 22 ...	106	27	133					
" 23 ...	179	54	233					
" 26 ...	275	168	443					
" 29 ...	205	156	361					
" 30 ...	244	81	325					
Aug. 5 ...	331	134	465					
" 7 ...	242	69	311					
" 9 ...	248	98	346					
" 10 ...	301	116	417					
" 12 ...	251	67	318					
" 16 ...	268	103	371					
" 17 ...	191	85	276					1
" 19 ...	148	28	176					
" 20 ...	244	69	313					
" 21 ...	212	47	259					
" 26 ...	391	130	521					
" 29 ...	225	50	275					
" 30 ...	119	33	152					
" 31 ...	161	42	203					

Date.	Unmarked flies caught.			Marked flies re-caught.				
	♂	♀	Total.	♂ 2 ♀	♂ 3 ♀	♂ 4 ♀	♂ 5 ♀	♂ 6 ♀
Sept. 4 ...	287	91	378					
" 7 ...	96	40	136					
" 9 ...	73	16	89					
" 10 ...	267	82	349					
" 12 ...	310	97	407					
" 14 ...	352	214	566					
" 17 ...	223	39	262					
" 19 ...	259	84	343					
" 20 ...	271	75	346					
" 21 ...	305	81	386					
" 23 ...	261	69	330				1	
" 24 ...	224	54	278					
" 26 ...	371	107	478					
" 27 ...	300	85	385					
" 30 ...	73	20	93				1	
Oct. 3 ...	451	121	572					
" 8 ...	255	28	283					
" 11 ...	292	51	343					
" 12 ...	269	37	306					
" 14 ...	303	89	392					
" 17 ...	287	53	340					
" 19 ...	274	65	339					
" 21 ...	434	69	503					
" 22 ...	389	95	484			1		
" 23 ...	362	65	427					
" 28 ...	314	54	368					
" 31 ...	415	132	547					
Nov. 1 ...	261	26	287					
" 4 ...	287	43	330					
" 5 ...	227	39	266					
" 6 ...	322	65	387					
" 7 ...	165	51	216					
" 9 ...	197	36	233					
" 11 ...	216	29	245					
" 12 ...	300	70	370					
" 13 ...	269	54	323					
" 18 ...	212	46	258					
" 19 ...	244	59	303					
" 22 ...	302	59	361					
" 23 ...	224	50	274					
" 25 ...	389	94	483					
Dec. 5 ...	304	33	337					
" 6 ...	349	54	403					
" 7 ...	291	108	399					
" 9 ...	210	42	252					
" 11 ...	284	33	317					
" 12 ...	287	53	340					
" 14 ...	247	121	368					
" 16 ...	388	99	487					
" 24 ...	509	138	647					
" 30 ...	271	77	348					
" 31 ...	330	49	379					

Date.	Unmarked flies caught.			Marked flies re-caught.				
	♂	♀	Total.	♂ 2 ♀	♂ 3 ♀	♂ 4 ♀	♂ 5 ♀	♂ 6 ♀
Jan. 3 ...	288	57	345					
" 6 ...	174	26	200					
" 9 ...	108	24	132					
" 10 ...	64	19	83					
" 11 ...	116	24	140					
" 15 ...	68	21	89					
" 16 ...	132	43	175					
" 17 ...	204	34	238			2		
" 18 ...	142	39	181					
" 20 ...	77	20	97					
" 21 ...	225	48	273					
" 22 ...	164	31	195					
" 23 ...	250	72	322					
" 24 ...	249	67	316					
" 27 ...	252	55	307					
" 28 ...	114	35	149					
" 29 ...	276	73	349					
Feb. 4 ...	211	60	271					
" 8 ...	465	104	569					
" 13 ...	347	66	413					
" 14 ...	376	71	447					
" 15 ...	352	59	411					
" 17 ...	208	55	263					
" 18 ...	270	43	313					
" 19 ...	175	22	197					
" 20 ...	153	17	170					
" 24 ...	300	50	350					
" 25 ...	210	49	259					
" 26 ...	75	21	96					
" 27 ...	71	22	93					
" 28 ...	186	32	218					

The last marked flies to be caught were noted on January 17, 1913. They were two *males*, in good condition, of the marking No. IV., and must have been marked sometime between April 1 and April 6, giving a minimum length of life of

247-253 days for a male fly.

The last marked *female* to be caught was noted on August 17, 1912, belonging to marking No. V.; hence marked between April 8 and April 13; this gives a minimum length of life of

126-131 days for a female fly.

It is of interest to note that the experiment done at Jinja (see *Progress Report*) gave a longevity of 182 days for a female, and of 149 days for the male.

Bruce has suggested (*Trans. Soc. Trop. Med.*, Vol. 5, No. I., p. 29, Nov. 1911) that, inasmuch as Lubbock kept a queen ant in captivity for years, the life of *Glossina* in the wild state may be as long as 2-3 years. But there can be drawn no analogy

between, on the one hand, an insect such as the queen ant, kept in an earthen nest under approximately constant conditions of temperature and humidity, and, on the other hand, an insect such as *Glossina*, of very different type, exposed to daily variations of temperature and humidity and dependent upon its own activity for a supply of food, which may not be constant.

On the mainland, at any rate, it seems probable that the fly will not live beyond the first dry season, which it encounters a few months after it has hatched out.

But on the lake margin, during the dry season, there is not sufficient diminution of humidity to destroy *all* flies for some may be found all the year through. It seems probable that these are flies which have hatched out shortly before the onset of the dry season, and are better able to resist adverse influences.

Conclusion.

A male fly has been re-caught 247-253 days after it was marked; a female fly 182 days after it had been marked.

INFLUENCE OF CLIMATE.

The question of longevity of the fly leads to the consideration of the effect upon it of climatic conditions. These will be shown to have a marked effect upon

1. The total numbers of the flies.
2. The proportions of the sexes.
3. The rate of larviposition.

The climatic conditions of Bugalla Island in the Sesse group contrast in several ways with those of the mainland, as will be seen from Chart I., which shows temperature, rainfall, and relative humidity at Bugalla and Entebbe from March 1912 to February 1913.

Chart I.

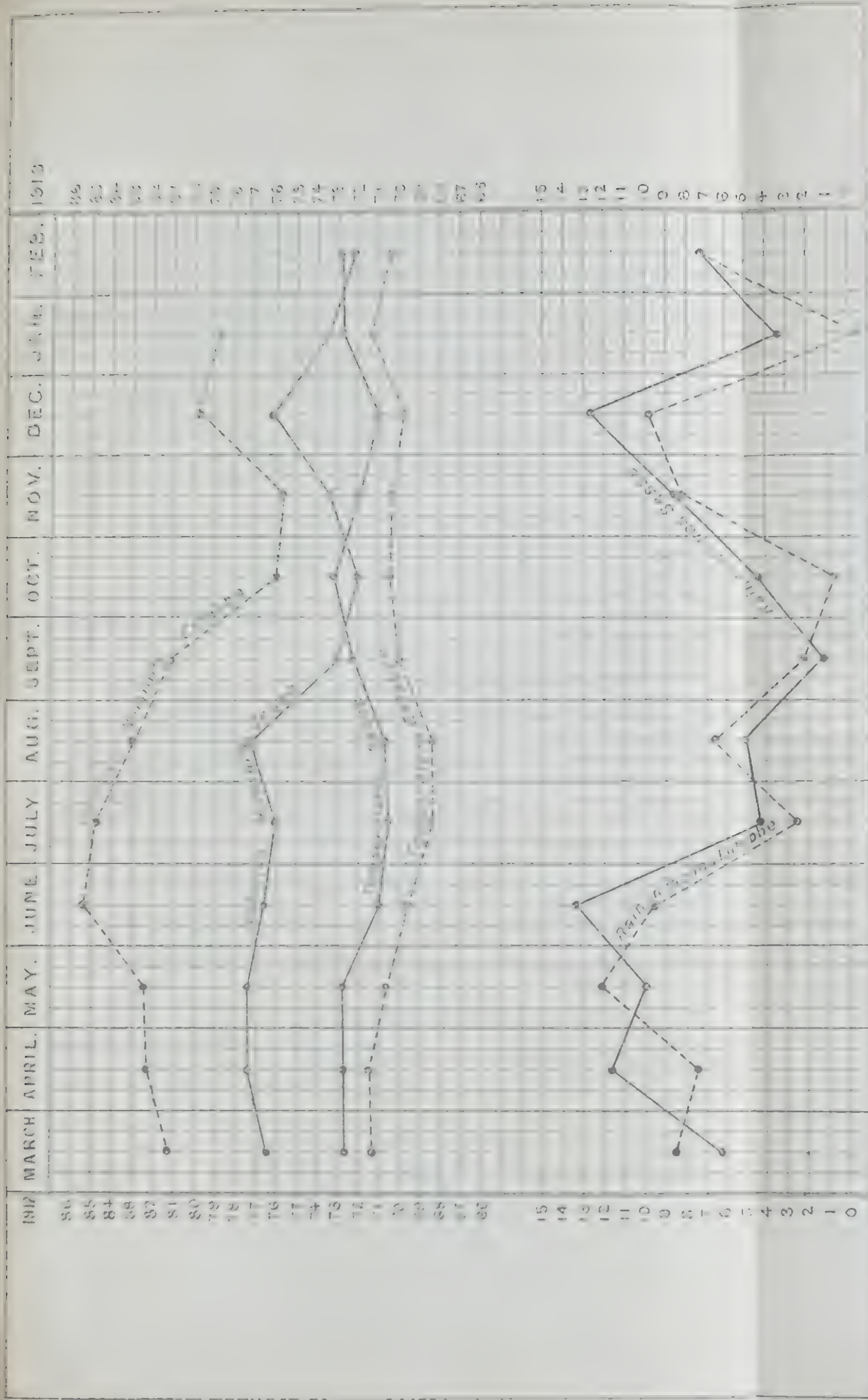
The Entebbe data are taken from the official gazette; the instruments with which the Bugalla records were obtained were lent by the Forestry Department at Entebbe.

The *temperature* and *humidity* figures for each month represent the average of the daily readings, each day's figure being the average of three observations taken at 7 a.m., 2 p.m., and 9 p.m.

The rainfall was read daily at 7 a.m.

The *temperature* on Bugalla was about two degrees higher than at Entebbe; this was presumably due to warmer nights owing to the slow cooling of the lake.

The relative *humidity*, however, was considerably below that of Entebbe. This was rather unexpected, but may be due to more constant wind on the island; the meteorological station at Entebbe is certainly not so exposed to the wind as was mine on Bugalla. The relative humidity was also much less variable on Bugalla, the difference between the greatest and least monthly figure being only half that at Entebbe. This is, of course, what would be expected on an island.



The *rainfall* varies from month to month, being sometimes greater at Entebbe, sometimes greater on Bugalla; the total for the twelve months, however, was greater on the island, being 89.62 inches against 76.52 inches at Entebbe.

I. Upon numbers.

1. *The influence of climate upon the numbers of the fly.*—In order to estimate truly the numbers of the fly it must be born in mind that they are sluggish on cold, cloudy days, and that few may then be caught, although there are in reality many present. So during the year's work, the days on which flies were caught were only such as would give a *true* estimate, and if, while the boys were catching, the sky became cloudy, they were called in.

Moreover, the catching was done during the same hours every day, viz., between 8.30-12; for the flies do not come readily to feed after mid-day until about 4 p.m.

It is obvious that, were flies caught in the morning one day and in the afternoon another day, no reliable results would be obtained.

Of course, reliance had to be placed upon the trustworthiness of the boys to catch conscientiously at the same rate every day; but by noting daily the number caught by each of the three, one soon knew whether an individual had fallen below his proper standard.

In order to make certain that the flies caught on one day were not the same as had been caught the day before (for all flies caught were liberated again at the close of the day's catching in order not to interfere with the natural course of events) every fly was marked, and *only those flies counted each day which had not been marked*.

Table I. shows the numbers caught.

If one looks at daily records, variations, coinciding with relative humidity, are sometimes very marked. For instance, during a period of relatively high humidity, when comparatively large numbers of flies are caught, a single day of diminished humidity will produce an astonishing difference (and vice versa), as in the following table:—

Table II.

Date.	Temperature.	Rel. humidity.	No. of flies per boy per hour.
1913.			
Jan. 8	72.7	74.7 per cent.	53.
" 9	74.	71.1	44.
" 10	73.3	61.	27.7
" 11	73.	71.1	46.7

Such daily differences I believe to be due to the varying avidity for food on the part of the flies; for it must be remembered that it is only the attractiveness of a fly boy as a source of food that leads to a fly being caught.

I have constantly found that, on days when there has been a little rain early, and then the sun shining through clouds, the flies are terribly persistent in their attacks, and it is impossible to avoid being bitten; whereas on a brilliantly fine, windy day the flies are not nearly so desirous of feeding.

I do not think, however, that this invalidates the conclusions to be drawn from the *monthly* averages.

The data obtained at Jinja in 1910 are here reproduced in a slightly altered form, the relative humidity having been calculated (*see* Chart II.).

From the curves it is at once obvious that there is a *direct* relation between the number of flies caught per fly-boy per hour, and the *relative humidity*, and an *inverse* relation between the number of flies and the *temperature*. This is the case on the *mainland* shore of the lake, where the variations in humidity are fairly large (*see* Chart I.). On *Bugalla Island*, however, there is no such obvious relationship; indeed, the number of flies caught seems rather to *coincide with the temperature* (*see* Chart III.). This may, I think, be explained by the much smaller range of variation of the relative humidity on the island (Chart I.) as has been already noted; so that a high temperature, with humidity not greatly diminished, is favourable rather than the reverse.

These facts will, I think, satisfactorily account for the great abundance of flies on the islands.

II. Upon sex proportions.

Secondly, climatic conditions affect markedly the proportions of the sexes.

In Charts 2 and 3 is shown, for Jinja on the mainland and Bugalla Island, respectively, the percentage proportion of females in the total number of flies caught each month, together with temperature and relative humidity.

There is, very clearly, an *inverse relation* between the *proportion of females and the temperature*.

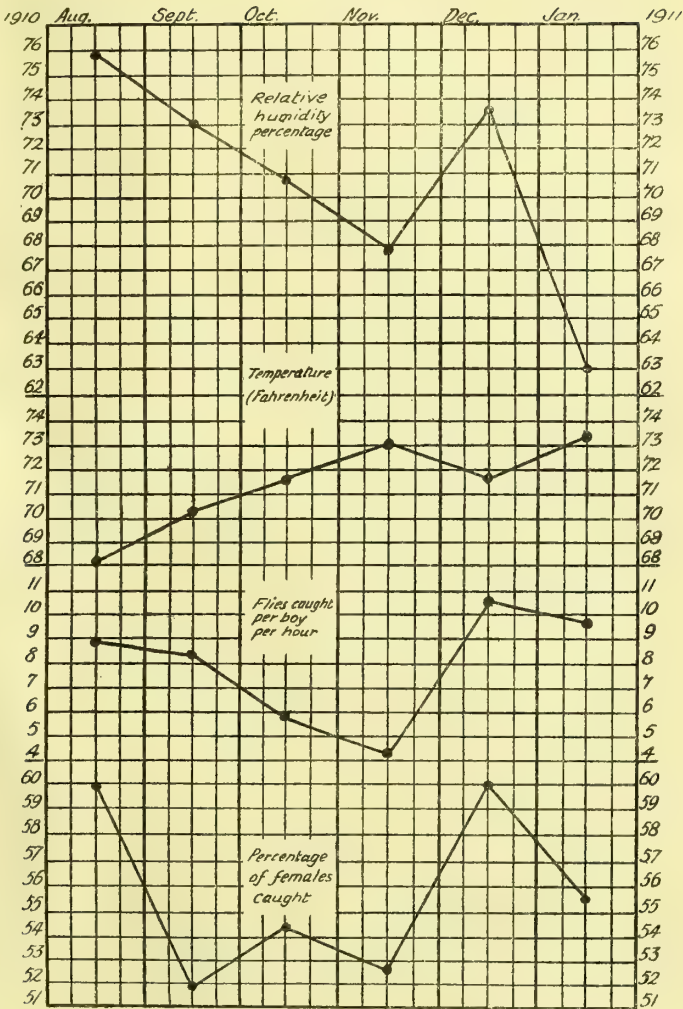
The correlation between the proportion of females and relative humidity seems to be, broadly, that obtaining between the total number of flies and the humidity, on the *mainland*. On the islands however, when the humidity varies less, there is no apparent relation. The inverse relation between the proportion of females and temperature suggests greater susceptibility of this sex to high temperatures, and *may account for the much smaller proportion of females on the islands*, where the temperature is higher (*see* Chart I.).

III. Upon rate of reproduction.

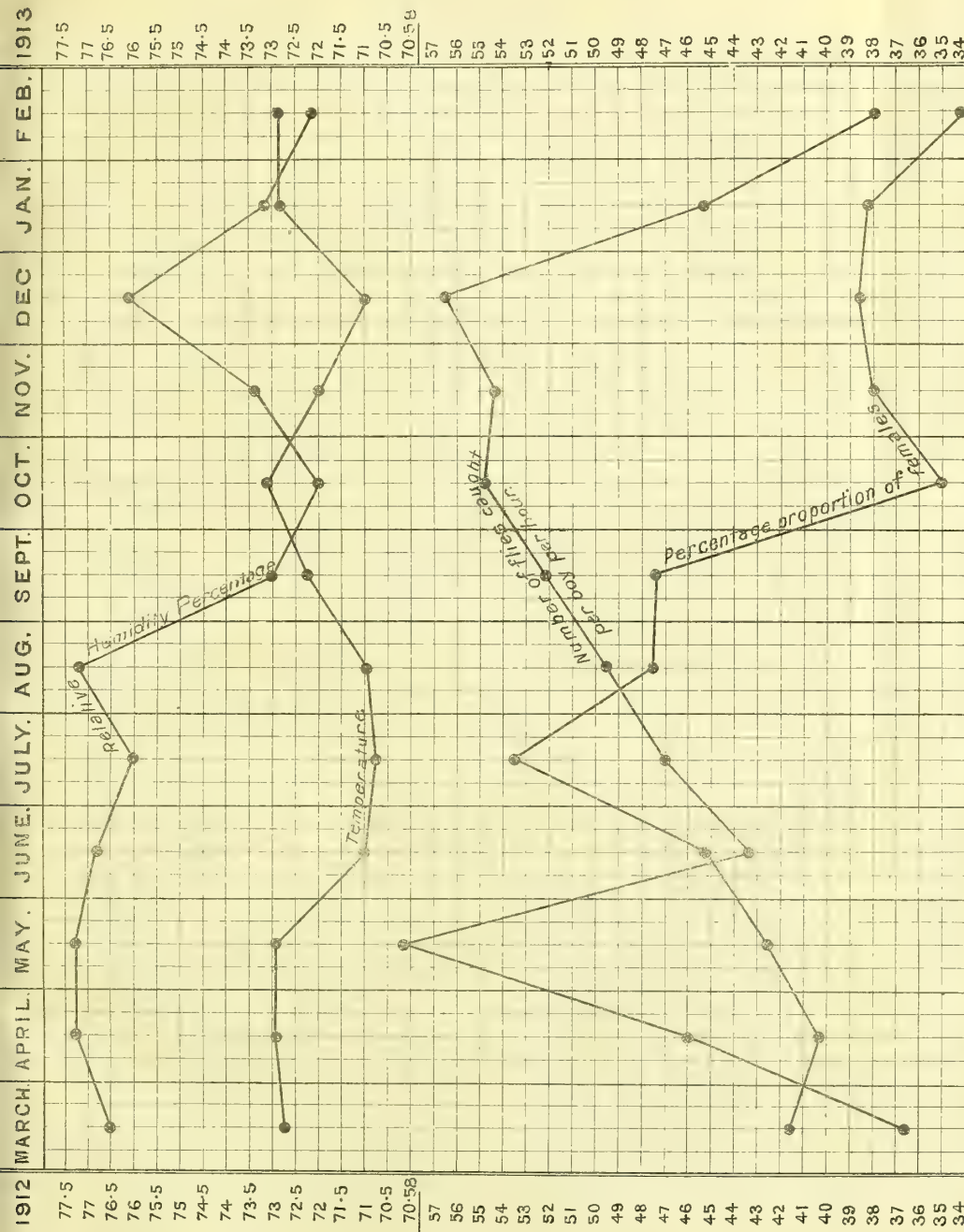
Thirdly, there is an intimate relationship between the number of larvae deposited and the relative humidity.

Pupae were looked for at the end of each month in the same localities by the same boys. Since a bonus was offered for each

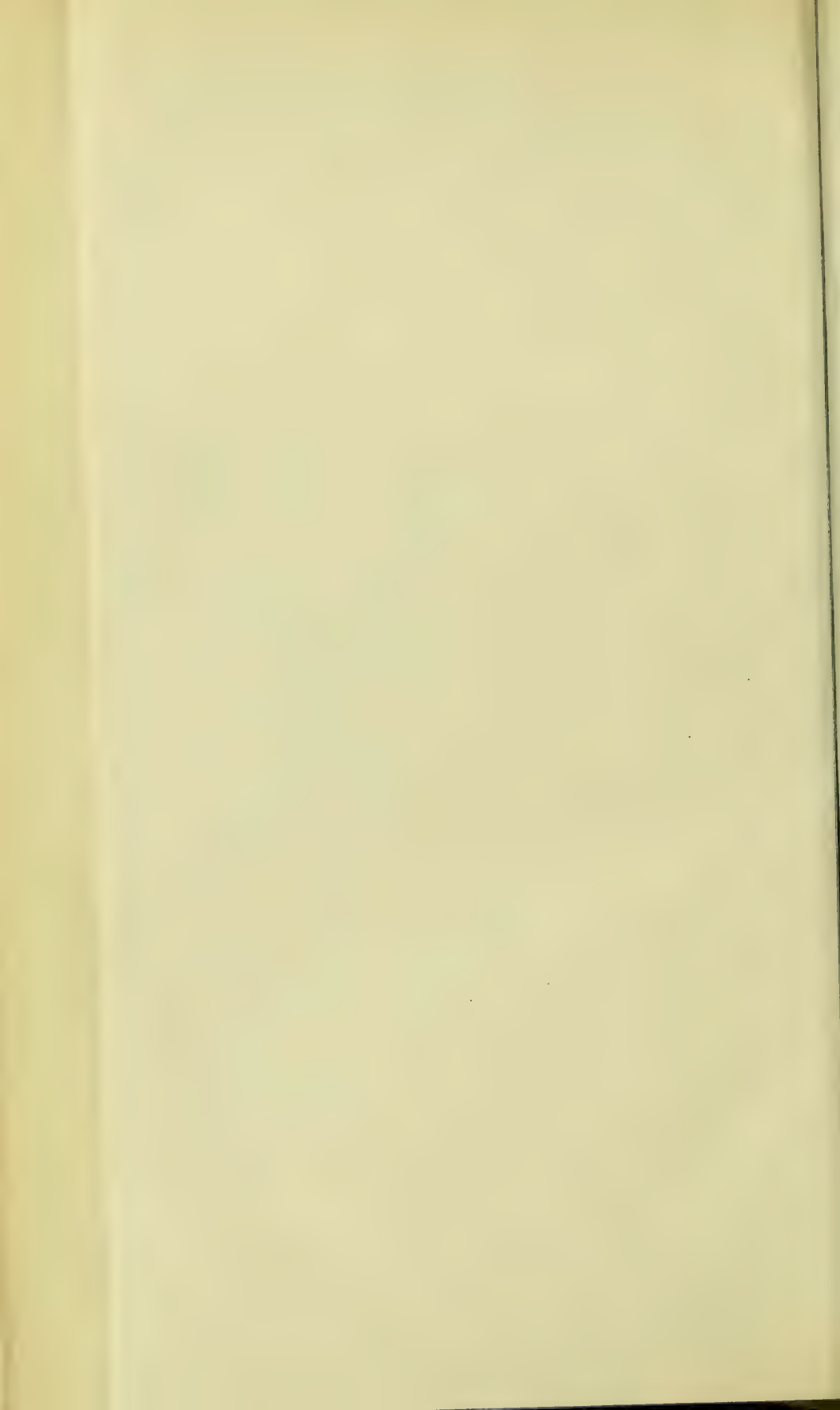
CHART II.

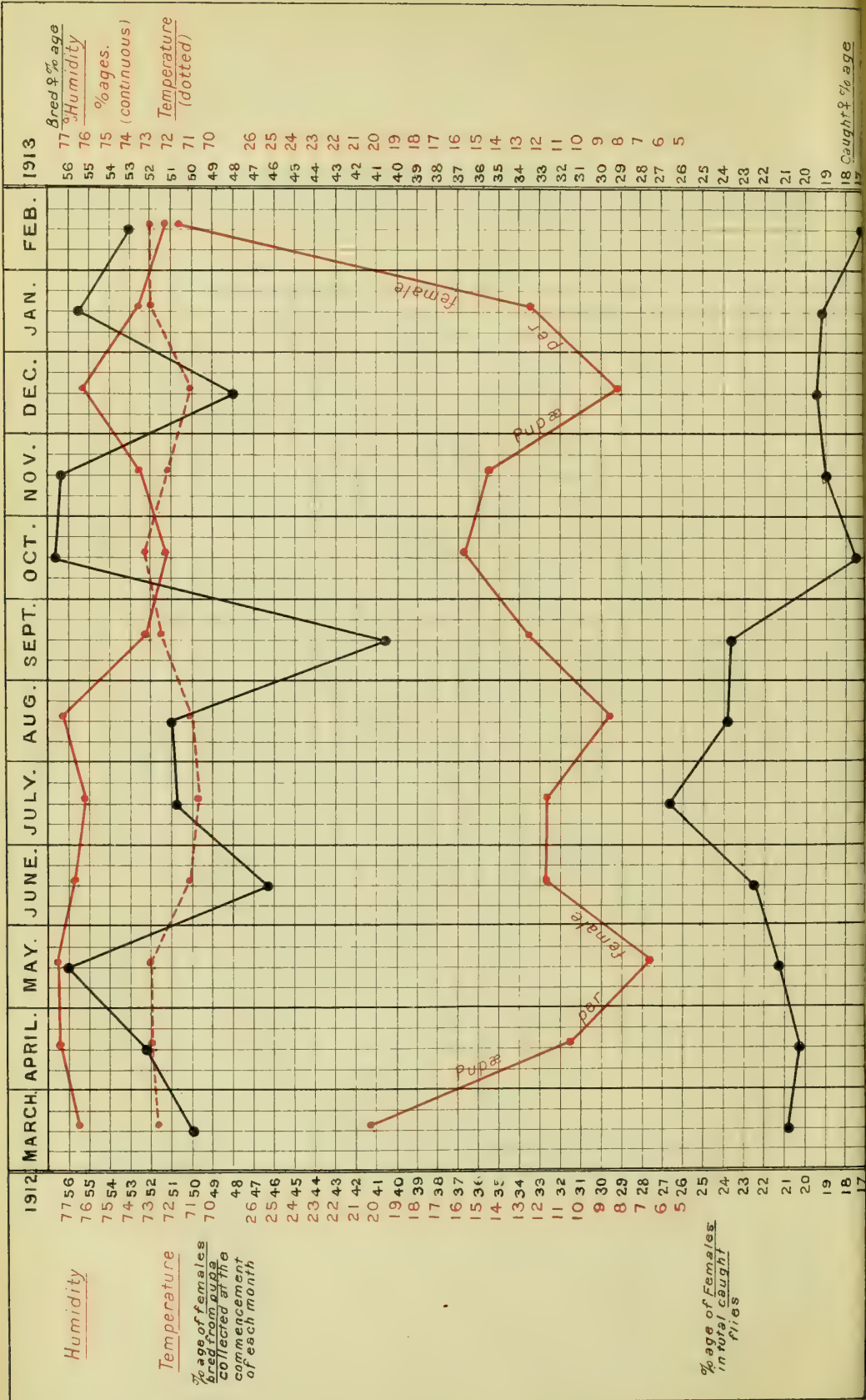


Influence of climate at Jinja, on the mainland



% age of Females. In order to fit the curve into the Chart the actual percentage has been multiplied by 12.5 so that the reading on the Chart must be halved to ascertain the correct number.





pupa there is every reason to suppose that the boys obtained all they could during the one morning on which they were sent to look for them.

It is obvious that the number of pupae found must depend, at least in part, upon the number of females to produce them, as well as upon the number produced by each female.

In order to estimate the ratio between the actual number of females and number of pupae, the average number of pupae found per boy at the end of each month was divided by the average number of females caught per boy per hour during that month.

For instance, the average number of pupae found per boy at the end of February was 187·3—the average number of females caught per boy per hour during that month was 6·3. The quotient obtained ($\frac{187\cdot3}{6\cdot3} =$) 29·7 is called the “Pupae per female.”

Though *in itself entirely meaningless*, it serves as a means of comparison between one month and the next.

On plotting this out it is at once obvious that there is a very definite relation between the “Pupae per female” curve and the curve of relative humidity: the relation being *inverse* (see Chart IV.).

This seems, in fact, to be a very pretty example of adaptation of the fly to the climate.

The pupal stage—in insects generally—is a stage of increased resistance to adverse conditions. Hence it has been brought about that the influences which are inimical to the adult fly cause an increased rate of reproduction whereby the race is preserved owing to the number of individuals surviving through the adverse conditions in a more highly resistant state. I have found that some of a batch of pupae can resist exposure of $\frac{1}{4}$ hour to full mid-day sun, naked on the lid of a tin box; this would be fatal to adult flies.

It seems clear, then, that the varying numbers of flies in wet or dry months may be explained partly by the effect on flies themselves and partly by the effect on the rate of reproduction. Flies are more numerous in months of greater humidity because during the previous months of diminished humidity reproduction has been more active; and also because the greater humidity is favourable to the flies themselves. Similarly, with opposite conditions, the effects are reversed.

Conclusions.

Climatic conditions effect *Glossina fuscipes* in three ways:—

1. Decreased relative humidity is unfavourable to the adult fly.
2. Higher temperatures are particularly unfavourable to the female sex.
3. There is an inverse relation between relative humidity and rate of larviposition.

I am very strongly of opinion that adverse climatic conditions are an extremely important, if not the main, check upon unlimited increase of the fly.

This leads to the question of the natural enemies of *Glossina*.

NATURAL ENEMIES.

Seing that, broadly speaking, the numbers of any species remain constant in a given area, it follows that of each generation as many must be exterminated as are produced, saving two which carry on the race.

Hence the number of offspring is directly proportional to the destruction thereof.

Now the rate of reproduction of *Glossina* is almost unique amongst insects; it is only matched amongst the other blood-sucking flies of the group Pupipara; no other insect, so far as I am aware, brings forth only one offspring at a time and only a total of a dozen or so.

This then is strong *a priori* evidence against any great destruction of *Glossina* taking place by the attacks of enemies. During the 2½ years of my investigations I have not succeeded in finding any enemies of importance.

I. Of larva.

Beside the ant mentioned in the Progress Report, another large black species was once seen on Damba Island carrying off a freshly extruded larva which had been picked up before it had had time to burrow. The identification of this ant has not yet been received, it appears to be as yet undescribed.

II. Of pupa.

No evidence has been obtained of any mammal, bird, or insect burrowing into or scratching up the loose soil of a breeding ground in order to devour the pupae.

No parasites have been reared from many thousands of pupae kept in closely shut boxes with glass lids.

It also seems doubtful whether the holes occasionally found in empty puparia (*see* Progress Report) are necessarily due to the exit therefrom of a Chalcid parasite. For on one occasion an empty puparium was found, from which the fly had emerged in the normal course of events; but there was *in addition* one of the small round holes such as those found in other puparia which have been supposed to be due to the exit of a Chalcid parasite.

Now, although an insect that *has* been parasitised *may* occasionally develop into the imago *after* the emergence of the parasites (as I have experienced with an Arctiid whose larva had been parasitised by a Tachinid), yet I do not think that *Hymenopterous* parasites ever fail to destroy the insects on which they have fed.

So that in this case it would seem, that, after the fly had emerged, some other insect had made a hole in the empty shell; it may possibly have been an ant.

Once, on Damba Island, a puparium was found with two such small round holes, and in it were several of an extremely small



FIG. 4.



FIG. 5.



FIG. 6.



FIG. 7.



FIG. 8.

FIGS. 4, 6, 8.
Lateral views.

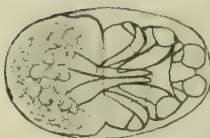


FIG. 9.

FIGS. 5, 7, 9.
Ventral views.



FIG. 10.
Empty Egg Shell.

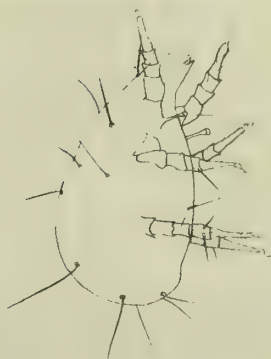


FIG. 11.

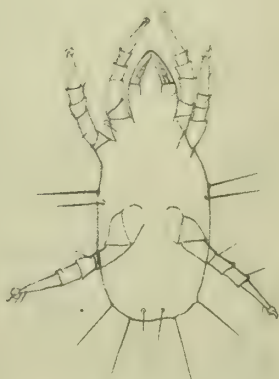


FIG. 12.

yellow ant. They ran in and out of the hole and seemed perfectly at home in the pupal shell, but when transferred to a glass tube with a fresh undamaged pupa, they made no impression upon it. During the last few months on Damba Island a large number of pupae in one of the boxes were found to be destroyed by an acarine, apparently of the family *Tyroglyphidae*.

At the same time, however, a large number of pinned insects in a box near by on the same shelf were found to have been similarly attacked; so it remains uncertain whether the acarines had been introduced with the pupae, or whether they were already on the pinned insects and had found their way to the pupae; and it is also quite possible that the pupae attacked were already dead.

Some freshly obtained pupae were placed in a tube together with a broken up infected pupa; but none were attacked. An infected pupa contained an enormous number of these little acarines, and their ova, in all stages of development, from which the accompanying drawings were made with a $\frac{1}{8}$ -inch objective.

Figs. 4-10.

The eggs are oval in shape, with a transparent shell, so that stages of development of the appendages can be readily made out. A large yolk mass lies at the dorsal aspect of the posterior extremity so that the egg floats in the fluid under the cover glass with this aspect uppermost, and it is difficult to get a view of one in the lateral position.

The young, 6-legged, larva has a curious structure arising from the ventral surface of the basal joint of the first pair of legs (see Figs. 11, 12). It appears as a stalk projecting ventrally at right angles to the limb and furnished at the extremity with an exsertile vesicle which may possibly act as a sucker to aid the larva at the 6-legged stage. As soon as the fourth pair of legs appears this structure is no longer seen (see Fig. 15).

Figs. 11, 12.

On one or two occasions, when examining preparations of the gut of a wild fly, I have seen the somewhat mangled integument of one of these acarines. It had probably been adhering to the exterior of the fly, and seems to suggest that the acarine may possibly attack *Glossina* in the wild state.

Figs. 13-15.

III. Of fly (newly emerged).

The fly, when it first emerges from the pupa, being soft and juicy, might be expected to fall an easy prey to enemies haunting the breeding ground for that purpose. I have, however, not been able to find any such.

On one occasion on Damba Island, when watching at the breeding ground, I saw a small Asilid fly preying on a soft, newly-emerged *Glossina*; this was the only occasion. I was unable to secure the Asilid.

One often finds that a locality that is suitable for larviposition is well suited for the pits of a species of "Ant lion" (the larva of *Macroleon polyzonus*, Gerst.).

It must sometimes happen that the freshly-emerged *Glossina*, as it hurriedly runs over the ground seeking for a vertical surface up which to climb in order to dry its wings, falls into one of these pits, which are often very close together, and is devoured by the voracious larva at the bottom. On one occasion I saw a freshly-emerged fly fall down into one of these pits, but the larva was apparently not hungry; it made no effort to catch the fly, and the pit being a small one the fly climbed out again.

IV. Of adult fly.

I do not think it likely that *Glossina fuscipes* is devoured by birds. It may well be that other species such as *G. morsitans*, frequenting more open country, are devoured by bee-eaters, drongos, &c. But the species which is the subject of this paper, frequenting as it does bush or forest with thick undergrowth, is out of the way of the larger insectivorous birds of powerful flight which would alone be capable of catching such an active insect. In my experience, bee-eaters, when found in the forest, are always high up on the tree tops, out of reach of the fly, or else frequent leafless shrubs in the middle of an open space where there are no fly.

I have shot two species: *Merops superciliosus*, which frequents tree tops in the forest, or bushes on the open shore, where it devours dragon-flies; and the smaller species, *Melittophagus meridionalis*, which frequents open grass land at the edge of the forest or tall reeds along the open shore, where there are very few bushes and consequently very few fly. Both species eat Hymenoptera very largely, and are particularly fond of dragon-flies.

Indeed, the relations which bee-eaters have to *Glossina* may be of two kinds. They devour quantities of the dragon-fly, *Cacergates leucosticta*, Burm., a species which I have on several occasions seen catching *Glossina*, so that in this way the birds are on the side of *Glossina*. On the other hand, they also devour a much larger species of dragon-fly, which itself preys upon *Cacergates*, so that in this way the birds are ranged against *Glossina*.

Prolonged observation in the forest belt at the back of the fly beach, haunted by fly, has convinced me that the birds found in it are not of the kind which could catch *Glossina*, with its phenomenal powers of flight. These birds are of the type which either flit about from bush to bush—such as weavers, wrens, small warblers, &c.—or sit upon a particular twig and dart upon an insect of slow flight, when on the wing, such as flycatchers, shrikes, &c., or pounce down upon large heavy insects on the ground, such as the orthoptera-eating kingfishers. There are of course large numbers of cuckoos and coucals (*Centropidæ*), but as they feed almost entirely on caterpillars they are out of the question.

Wagtails, which abound on the sandy shore, and swallows, confine themselves to small insects as a rule, though wagtails may sometimes be seen devouring a large but *easily-caught* insect

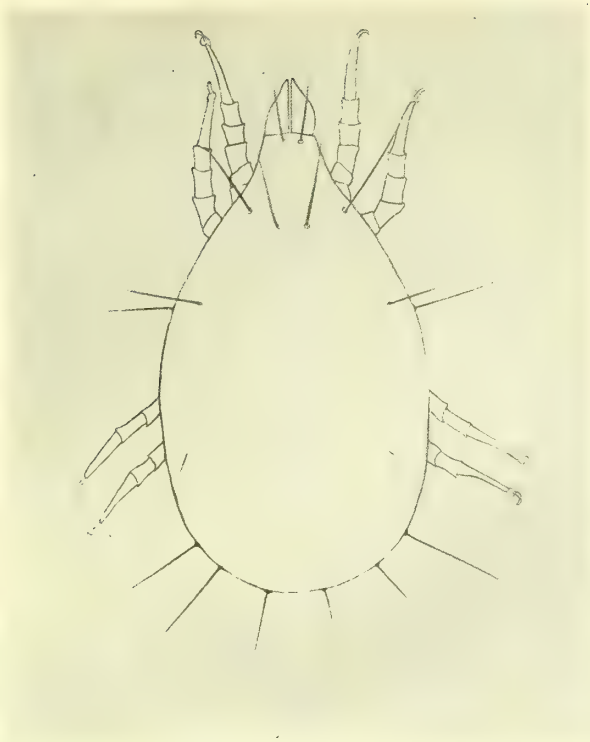


FIG. 13.



FIG. 14.

The following table gives the result of examination of the stomach contents of 64 insectivorous birds shot while feeding in, or on the borders of, the fly areas of Damba and Bugalla Islands, since the Progress Report was written. In that it was mentioned that 26 bee-eaters and 26 other birds had been examined; making 116 in all with negative result.

Table III.

No.	Date.	Locality.	Bird.	Stomach contents.
1	26.9.11	Damba jungle.	Black and white fly-catcher.	Very small insects.
2	"	"	Chestnut, black, and white long-tailed fly-catcher known as "Kunguvu."	" "
3	14.10.11	On shore, Damba.	Merops superciliosus	Mainly dragon flies.
4	"	"	Melittophagus meridionalis.	" "
5	16.10.11	"	Merops superciliosus	Dragon flies and honey bees.
6	"	"	" "	Very "small insects."
7	"	"	Small grey flycatcher	" "
8	17.10.11	"	Warbler ...	" "
9	26.10.11	"	Small grey flycatcher	Medium-sized parasitic Hymenoptera.
10	2.11.11	"	Merops superciliosus	Dragon flies and honey bees.
11	"	"	" "	" "
12	"	"	Grey flycatcher ...	An Agrionid dragon fly and other smaller insects.
13	11.11.11	"	Merops superciliosus	Dragon flies and bees.
14	"	"	Chat ...	Ants and Forficulidæ.
15	13.11.11	"	Merops superciliosus	Dragon flies and bees.
16	"	"	" "	" "
17	"	"	" "	" "
18	14.11.11	"	" "	" "
19	"	"	" "	" "
20	"	"	" "	Dragon flies, bees, and a species of Belonogaster.
21	"	"	Melittophagus meridionalis.	Dragon flies and bees.
22	"	"	Grey flycatcher ...	Mainly small winged ants.
23	"	"	" "	" "
24	24.11.11	"	Small red brown shrike	Grasshoppers.
25	"	"	" "	" "
26	"	"	" "	and one beetle.
27	30.11.11	"	Merops superciliosus	Dragon flies only.
28	"	"	" "	" "
29	"	"	Melittophagus meridionalis.	" "
30	"	"	" "	" "
31	"	"	" "	Dragon flies and a small lamellicorn beetle.
32	9.12.11	"	Merops superciliosus	Dragon flies and bees.
33	"	"	" "	" "
34	"	"	" "	" "
35	"	"	" "	Dragon flies.

No.	Date.	Locality.	Bird.	Stomach contents.
36	9.12.11	On shore, Damba.	Melittophagus meridionalis.	Winged ants and one small dragon fly.
37	"	"	"	Dragon flies and bees.
38	11.12.11	"	"	Winged ants only.
39	"	"	"	Winged ants and one Agrionid dragon fly.
40	"	"	"	Winged ants and remains of a small chrysidid.
41	"	"	"	Winged ants.
42	"	"	Merops superciliosus	Dragon flies and bees.
43	13.12.11	"	"Kunguvu" flycatcher	Two large Hymenoptera parasitica.
44	16.12.11	"	Small flycatcher ...	Indistinguishable minute insects.
45	22.1.12	Bugalla flyground.	"Kunguvu" flycatcher	" "
46	30.1.12	"	" "	" "
47	9.3.12	"	" "	" "
48	"	"	" "	" "
49	13.3.12	"	Small black and white flycatcher.	Green caterpillar and minute insects.
50	26.3.12	"	"Kunguvu" flycatcher	Indistinguishable minute insects.
51	5.4.12	"	" "	Semi-digested mass, probably caterpillars.
52	"	"	Grey flycatcher ...	Indistinguishable minute insects.
53	27.9.12	"	"Kunguvu" flycatcher	Six Agrionid dragon flies, six small caterpillars, a small cocoon, one or two beetles.
54	"	"	Bronze green cuckoo	Caterpillars only.
55	30.9.12	"	"Kunguvu" flycatcher	A single large Noctuid moth, about two inches in expanse.
56	"	"	" "	A mass of small insects—remains of several small Hymenoptera (? Sawflies) and a beetle could be seen.
57	19.10.12	"	" "	Indistinguishable remains, save for one small spider.
58	"	"	" "	" "
59	21.10.12	"	" "	" "
60	25.11.12	"	" "	Remains of Hymenoptera—all of apparently the same species, but too much broken up for identification.
61	25.11.12	"	Merops superciliosus	Caterpillars and young mantids.
62	14.12.12	"	" "	Eleven honey bees.
63	"	"	" "	Twelve honey bees, one dragon fly, one wasp.
64	"	"	" "	Twelve bees, one ♂ Paltothyreus tarsatus.

N.B.—Up to No. 53 the examination was by naked eye; from 53 onwards by a low power microscope.

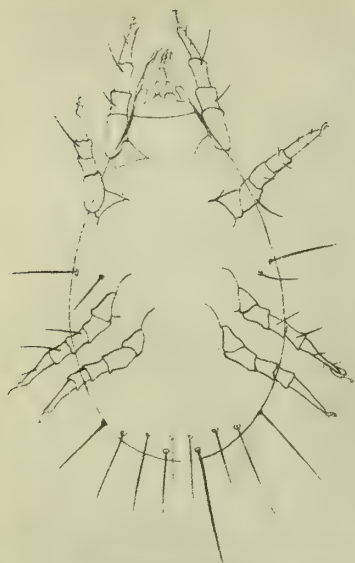


FIG. 15.

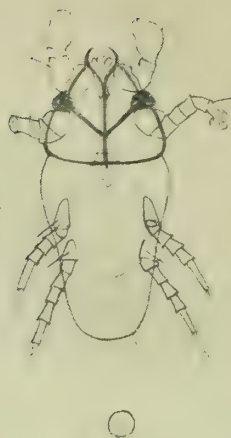


FIG. 16.

A human erythrocyte below
on same scale.



FIG. 17.

It was mentioned in the Progress Report that the dragon fly *Cacergates leucosticta* had been seen to catch and devour *Glossina*. This is an extremely abundant species, and if one walks along the shore one is at once surrounded by a crowd of these insects, some flying backwards in front of one as one walks, and all apparently on the look-out for *Glossina* which they frequently attempt to catch; and on several occasions they have been seen to do so.

Dragon flies.—On one occasion in the early morning a young hippo was on the fly beach, and I was able to approach closely enough to see with glasses numbers of *Glossinæ* on its flanks and buzzing round it. Very many dragon flies were in attendance, and could be seen darting at the *Glossinæ*, and I have no doubt that they account for a certain number of full fed flies which are relatively heavy on the wing.

Cacergates leucosticta, unlike many dragon flies, is not dependent upon the sun, but is active at all times of day, even before sunrise and after sunset, whether cloudy or bright.

As has been said, this is a favourite food of bee-eaters, and is often also preyed upon by larger dragon flies which are themselves devoured by bee-eaters.

Another species of dragon fly, identified by the Entomological Research Committee as *Orthetrum farinosum*, Forst., was seen in Damba Island forest to catch and devour a *Glossina* from off my clothes. A male was first observed to do this, and later, a female.

Asilidæ.—The powerful and very active predaceous flies of the family *Asilidæ* might be expected to be important enemies of *Glossina*, since their appetites are apparently insatiable and they prey upon insects of all kinds, even the most active falling victims. But although I have spent many hours watching in suitable places at the edge of the forest or in open sunny spots in the forest (localities frequented by *Asilidæ*), where *Glossina* abounded, an *Asilid* has only once been seen preying upon *Glossina*.

The species has not yet been identified, but it was not a large one, being very little larger than *Glossina* itself.

Acarines.—The red *Acarines* mentioned in the Progress Report, which are frequently found attached to *Glossina*, have been reported on by the Entomological Research Committee as being all larvæ, and probably only making use of *Glossina* as a means of transport.

On Bugalla Island a thousand flies have been dissected and their gut contents examined, and on several occasions very minute acarines have been found in the preparations. Seeing that, though minute, they were too large to have been ingested through the proboscis, and that they were probably not *internal* parasites, it seems most probable that they had been adherent to the integument and had been scraped off in the process of removing the gut. Fig. 16 shows one of these.

The dorsum of the cephalothorax is marked out by strongly chitinised lines into four plates, between which, at the side, is a thick mass of chitin, and the side of the anterior process which encloses the mouth parts is also strongly chitinised.

Folded ventrally, in the mid line, appears to be a long rostrum(?) with bulbous transversely striated extremity, but this was extremely difficult to make out. A large sac-like organ could be seen lying between the two posterior pairs of limbs.

The anterior pair of limbs only had four joints, the terminal one being swollen. The second pair terminated in a pair of claws and a pulvillus; these could not be seen on the two posterior pairs.

Fig. 17 shows another acarine found in a preparation from the gut of a female fly.

Fig. 18 shows another Acarine, found in a preparation made from the mid gut of ♂ fly Sesse 838. Obj. $\frac{1}{6}$.

Drawn with camera lucida eyepiece on a somewhat larger scale than the two former drawings.

The mouth parts could not be deciphered; they were represented by two very dark opaque rounded masses. The two anterior pairs of limbs terminated in a membrane with a hooked tip. The third pair were extremely short and clubbed. The fourth pair also short, but with an extremely finely-drawn-out terminal appendage.

In one fly on Damba (♀ 74) the integument of an Acarine was found in a gut preparation. The Acarine was apparently of the same species as that mentioned as having been found in pupae, and had possibly been adherent to the integument.

Nematodes.—It was mentioned in the Progress Report that a larval nematode had been found in a gut preparation from a bred fly. An adult helminth had not then been met with; since then four have been met with in the abdominal cavity of as many flies—over a thousand flies having been examined. The fly seemed in no way inconvenienced by the presence of the parasite, which was at least two inches long. In one or two cases the fly had just had a full meal of blood.

Bacilli.—It was mentioned in the Progress Report that bacilli had been found in 19 per cent. of the wild *Glossina*.

This is much below the mark. If the gut be divided into fore-, mid- and hind- portions (the fore gut being all that behind the thoracic gut and in front of that part which usually has green contents, and in which the blood when present maintains its bright red colour), the forepart will be found almost in every case to contain bacilli in varying number—sometimes literally in masses.

When this is the case the gut wall seems thin and friable, and it is possible that the fly is sickly.

Bacilli are similarly present in almost every bred fly before it has had a feed or been let out of the breeding box.

Microsporidiosis.—H. B. Fantham has suggested (*Trop. Dis. Bulletin*, Vol. I., No. 3, December 15, 1912, p. 157) that if a microsporidium could be found as pathogenic to *Glossina* as *Nosema apis* to the hive bee, the discovery might be put to practical use.

This suggestion, however, seems to have been made without due consideration of the important differences between *Apis* and *Glossina*.



FIG. 18.

Camera lucida drawing, on much larger scale than
the other drawings.



The bee contracts infection by the ingestion of food soiled by excrement from another infected bee; or by fouling its mouth parts with excrement from an infected member of the hive. *Glossina*, however, can never infect itself by ingestion of infected food, seeing that its food is obtained by suction after piercing the surface.

Moreover, it does not void any of the superfluous fluid which it has ingested until after it has finished feeding, and has flown away to rest on a shady spot near by; so that there can, practically speaking, be no chance of its ever fouling its proboscis with excrement from an infected individual, and it is difficult to see how, should one *Glossina* be infected, it could infect another, save in the act of mating.

Suppose, however, that such a pathogenic Protozoon, transferable from one sex to another, could be found, and that it was infective to *Glossina*. If it is to be of any practical value, it must at least render the infected fly so sickly that it dies of starvation (*i.e.*, is unable to transmit the trypanosomes of sleeping sickness). In this case, the fly would be rendered *incapable of mating* (a process which can be seen in the field to demand the utmost activity on the part of the male, which, as it were, captures the female by force) and the parasitic Protozoon would perish together with the individual it had infected. Still more would this be the case if it were of sufficient virulence to kill the fly outright.

There seem, then, to be very strong theoretical difficulties in the way of any practical use of pathogenic protozoa.

Fungoid disease.—Out of many thousands of wild flies that have been caught and handled, none have shown any signs of infection with a fungus, such as *Empusa muscæ*, which destroys the house fly in large numbers. If it is true that the spores do not infect the fly from without, but only when *swallowed*, the same difficulty is in the way of the practical use of *Empusa* as in the case of Protozoa.

Conclusions.

In addition to those which have been recorded in the Progress Report, the following insects have been observed to attack *Glossina*:—

- I. *Larva*. An ant. Species not known.
- II. *Newly emerged fly*. An Asilid. Species not known.
- III. *Adult fly*. An Asilid. Species not known. A dragon fly (*Orthetrum farinosum*).

No evidence has been obtained that birds devour *Glossina* in any stage; it does not seem probable that they do so.

Sundry Acarines have been found which were possibly external parasites.

Nematodes have been found in the abdominal cavity of four out of over a thousand flies.

FOOD SUPPLY.

The question of the *Food Supply* of *Glossina* can be subdivided under three heads.

- I. The sources from which it derives blood.
- II. Whether it feeds on vegetable juices.
- III. Whether it sucks up water.

I. *Blood*.

The blood found in wild flies may be considered firstly as Mammalian (M), or Non-mammalian (N).

Occasionally M and N may be found together in one fly, which had presumably been disturbed at one meal and gone on to another.

The percentage proportion of M and N at the three localities in which I have worked is as follows:—

Table IV.

Date.	Locality.	No of flies examined.	No. of flies in which nature of blood recognisable.	M %.	N %.
Nov. 7, 1910 to Feb. 10, 1911.	Jinja (mainland)	600	92	31.5 %	68.5 %
March 23, 1911 to Dec. 18, 1911.	Damba Isle.	695	177	15.2 %	84.8 %
Jan. 12, 1912 to Jan. 14, 1913.	Bugalla Isle.	1,000	246	68.7 %	31.3 %

It is interesting to see how the proportions of M and N on Bugalla Island are exactly the reverse of those of the mainland.

The excess of M on Bugalla is very probably to be explained by the great abundance of Speke's *Tragelaph* (*Situtunga*), which is to be found throughout the day resting in the forest belt along the shore where flies are numerous, and in the evening, as early as 4 p.m. coming out and feeding at the edge of the forest which is just the time when *Glossina* feeds voraciously and in just those localities. I am certain that the *Tragelaphs* were more within reach of the fly on Bugalla Island, Sesse, than on Damba Island.

Fig. 2 (p. 2) shows a typical locality of this kind, where I have many times seen *Tragelaph* in the late afternoon and early evening; they come up through the forest belt which runs up the hill side from the water's edge, and feed at the border of the forest and grass—and the flies are very numerous in just such a locality.

As regards *Hippopotami*. I have seen one on the beach with many flies on his flanks, and buzzing round; but I think the fly on Damba Island had just as many facilities for feeding from hippos as on Bugalla Island, Sesse; so that the difference in the proportions of M and N in the two islands is probably due to the *Tragelaph*.

The *otters* which abound in the lake must not be overlooked as a source of mammalian blood. When resting on the sand or rocks by the water, they must be very attractive to *Glossina*, which is always particularly attracted by a wet animal.

If one examines flies daily, and notes the proportions of M and N, one is at once struck by the fact that from day to day the proportions are very different. If, for instance, there are fresh tracks of *Varanus* (the large monitor lizard known in Uganda as "Nswa-swa") on the sand when flies were caught, the proportion of N to M of that day is much greater than the average. This shows with what eagerness the flies avail themselves of any source of food which presents itself; and also, that *it is quite impossible to gauge correctly the proportions of M to N from one or two casual examinations in any particular locality.*

The proportions of M to N were found to vary considerably from one month to another, but when the results were plotted out in a curve, and compared with the curves of temperature, humidity, number of flies, etc., no correlation whatever could be seen, and the variations appeared purely fortuitous. Table V. shows these variations in flies caught and examined on Bugalla Island in 1912.

Table V.

Month.	No. of flies in which blood recognisable as M. or N.	M. %.	N. %.
March	9	71·4	28·6
April	20	75·	25·
May	24	58·3	41·7
June	16	42·8	57·2
July	13	76·9	23·1
August	23	72·7	27·3
September	21	63·7	36·3
October	27	60·6	39·4
November	36	66·7	33·3
December	29	74·2	25·8

It would be of interest to examine a sufficiently large number of flies every month so that enough data could be obtained to show in the case of the proportions of M and N *in females alone*, whether there was any correlation between the rate of reproduction and the proportions of M and N; *i.e.*, whether during the times when reproduction is more active, the female requires more blood of a certain type which may be better suited for the development in utero of the larva.

My data are not sufficient for this—a very large number of flies would require to be examined every month. For, firstly, the percentage of females in the total number of flies is very small (at least, on the islands), secondly, of these, only a few will contain blood, and thirdly, of these, in only a certain number will the blood be sufficiently recent to be ascribed to its source. The sources from which non-mammalian blood may be derived are, theoretically, numerous.

A. *Non-mammalian.*

Birds, reptiles and amphibians might all be drawn upon of *Birds*. The species most likely to supply blood to *Glossina* are cormorants and darters, which sit at the water's edge on rocks or bushes, often with wings widely outspread, so that it would be easy for flies to bite in the axilla or on the under surface of the wing, where feathers are sparse. On one occasion, I took a cock to the fly ground and tethered it by the leg and watched it for a couple of hours. Although flies were numerous and settled freely on my clothes, none were seen to make any attempt to feed on the cock, although the comb and wattles offered a free supply of blood. It is extremely rare to find avian blood in wild flies as will be seen below.

Reptiles, which may supply food to *Glossina*, are numerous. Crocodiles are well known to be important in this connection, but it is not, I think, sufficiently realized that the large monitor lizard, *Varanus*, which haunts the water's edge, is of equal importance; probably more so, as it is extremely numerous since the natives who hunt it for its skin have been withdrawn from the lake shore. On several occasions, flies have been actually seen swarming on a *Varanus*; sometimes the top of its head was literally black with them—this was especially the case when it was lying half-covered in the water, or gently swimming along the edge with the top of its head uncovered. As will be shown later, its blood is frequently found in flies.

It seems possible also that smaller lizards may be bitten, as will be seen when the measurements of blood cells are discussed.

Snakes may also be fed upon, though no positive evidence has been obtained of this. But one would think that a large python, lethargic after a meal, must be of considerable value as a source of food. On one occasion, I came upon a large black cobra coiled up in the forest in a sunny spot; and it seemed to me that there was an unusual number of flies collected there. The snake disappeared at my approach, so that I could not determine whether the flies were actually feeding.

Tortoises.—On two occasions a species of tortoise frequenting the edge of the lake has been seen with numerous flies running on its shell, obviously eager to feed. Doubtless, if they bit upon a soft part (for the legs and tail are unprotected by the shell), they would be able to feed, and they certainly were desirous of doing so. I may say that I came upon the tortoise quite suddenly, so that there can have been no doubt that the flies were there already, and had not come with me.

Amphibia do not appear to be fed upon, although green frogs abound in the fly area on the low vegetation. No amphibious blood has been found in the series of flies examined.

The *Lung fish* (*Protopterus*) is not likely to be fed upon *in the lake*; though what may happen in streams which dry up in the dry season is another matter. But its corpuscles are so *enormous* (see below, Table 7), that only one at a time could be drawn up the proboscis, there not being room for two abreast, as has been proved by measuring camera lucida drawings of both on the same

scale. This would make the suction of such blood a matter of extreme physical difficulty, even should the fish present itself to the fly for a long enough period. In order to obtain an estimate of the relative values of these different sources of N, camera lucida drawings were made of corpuscles found in wild flies, and the measurements subsequently compared with those from corpuscles of known origin.

In Vol. XI. of the Reports of the S.S. Commission, pp. 112-119, is a paper by Bruce, Hamerton, Bateman, and Mackie, on this subject, in which the authors find that out of 20 flies, seven had probably fed from a bird, 13 from reptile or amphibian. The paper, however, is open to criticism on the following grounds:—

- I. Only "10 to 20" red cells were measured. Seeing that in the blood as obtained from a fly, one corpuscle may be half as large again as another, though both may have preserved their natural shapes, the number is too small to permit of a fair average being obtained.
- II. Only the length was measured. If the ratio of breadth to length be taken into account, additional aid is afforded in the differentiation of corpuscles.
- III. A single type of blood, that of the crocodile, was taken as a standard of all reptilian and amphibian blood. It will be shown below that there is much variation in the measurements of blood from these sources.
- IV. As a standard of avian blood, the *Hornbill* was taken; a bird which frequents the tops of the trees in the forest, and can hardly ever be within reach of *Glossina*.
- V. "No allowance could be made for alterations due to digestive changes."

It will be shown below that this *is* possible.

The method of procedure was as follows. The blood having been stained in a film by Leishman's method, and a preliminary examination having shown that it had not been so far altered by digestion that unaltered corpuscles could not be found for measurement, drawings of 50 corpuscles were made with Leitz camera lucida eyepiece, and $\frac{1}{12}$ inch objective, always on the same scale.

The corpuscles were drawn as they came into view, only providing that their shape and proportion were unaltered. There is considerable variation in the *size* of such, which is probably due to the fact that the effect of a sojourn in the fly's alimentary canal is shown more quickly by the corpuscles on the exterior of the mass. But if a large number be drawn, a fair average can be struck.

Since all the drawings were made on the same scale, all that was necessary was to compare the average measurements of the drawings with those of types treated in precisely the same manner. For the purposes of comparison there was no need to go into the question of the *actual* size of the corpuscles. The most perfect method of comparing types with the unknown would be to feed flies on the type, so that the blood in both cases would have been subjected to the same process.

Unfortunately, it was only found possible to feed flies on a cormorant—they could not be induced to feed on a captive varanus—the reason probably being that it had been kept during the night, and the flies were mistakenly put on in the morning before it had warmed up in the sun.

No captive crocodile was available.

In order to estimate the effect of digestion, flies were fed on a cock, and the blood from them examined at intervals afterwards, and the drawings compared with drawings of blood obtained by pricking the cock's comb.

It was found that up to about 30 hours after the feed, the corpuscles in the fore part of the gut retain their shape, but that after that they swell up and lose their characteristic proportions. Until they do this, and so long as they retain their shape, they shrink slightly; this presumably is due to the very rapid evacuation by the fly of the excess of fluid which it has ingested.

The following Table VI. gives the results obtained from the cock's blood, the measurements being in *millimetres*, representing the average size of the drawings of 50 corpuscles from each fly.

Table VI.

Time after fly fed.	Length in millimetres.				Breadth in millimetres.				Ratio of Breadth to Length.	Time after fly fed.
	Average.	Max.	Min.	Range of variation.	Average.	Max.	Min.	Range of variation.		
1 hr.	18.06	24.	10.25	13.75	10.64	13.25	7.5	5.75	58.2%	1 hr.
2 hrs.	18.3	23.75	14.5	9.25	11.06	14.5	8.75	5.55	60.4%	2 hrs.
3 hrs.	19.26	24.	14.	10.	10.76	14.75	8.5	6.25	55.8%	3 hrs.
5 hrs.	18.55	24.75	14.15	10.25	10.09	13.	8.25	5.75	54.4%	5 hrs.
7 hrs.	18.46	24.75	14.	10.75	10.34	13.	8.25	4.75	56.	7 hrs.
30 hrs.	18.23	23.75	15.	8.75	10.	12.	8.25	3.75	54.1%	30 hrs.
Direct from cock	19.16	24.25	15.5	8.75	11.29	13.5	9.25	3.25	58.9%	Direct from cock.

From this it will be seen that in all cases save one (the 3 hours fly) there was slight shrinkage shown; in the exception, however, there was an infinitesimal *rise*, which may fairly be put down to experimental error.

It appears also that the *range of variation* in size of the corpuscles is greater in the case of blood taken from the fly than in normal blood, bearing out what has been said regarding the greater effects on the corpuscles on the outside of the mass.

It appears, then, that when one is comparing an unknown blood with a series of types it cannot be ascribed to an animal whose corpuscles are, even slightly, *smaller* than that of the specimen under consideration, and must be put under a type whose corpuscles are of the same size or slightly *larger*, and agree more or less in the breadth to length ratio.

In order to estimate the *actual* size of the corpuscles in micro-millimetres (μ), since the magnification used was not known, the normal human erythrocyte was used as an index, the blood being taken from the writer.

Fifty corpuscles were drawn on exactly the same scale as the others, and the average diameter of the drawings calculated. It was found to be 11.77 millimetres.

Then taking the average size of the human erythrocyte to be 7.5μ , 11.77 millimetres of the drawing equal 7.5μ , so that 1 millimetre of the drawing equals $\frac{7.5}{11.77} = 0.637 \mu$, the factor with which to reduce the measurements of the drawings to the actual size of the corpuscles.

The following table gives the sizes, so estimated, in micro-millimetres of sundry types of blood arranged in descending order of size.

Table VII.

—	Length in micromilli- metres.	Breadth in micromilli- metres.	Breadth-Length Ratio percentage.
Protopterus ¹	41.5	27.7	66.8
Tortoise	18.6	10.6	56.9
Frog ²	17.3	10.9	62.9
Python ³	16.7	9.9	59.2
Crocodile	? 15.8	? 9.4	? 53.2
Varanus	15.7	8.6	50.9
Brown Lizard... ..	14.4	7.2	50
Cormorant, ⁴ from fly 1 hour after feeding.	12.1	6.6	54.1
Do. do. 2 hours	11.2	6.4	57.1

As regards the *crocodile's blood* I am a little doubtful if the measurements given accurately represent the size. I was unable to obtain a specimen myself and the specimen sent me was very dirty and not a good one, and I had some difficulty in finding suitable corpuscles from which to obtain measurements. Moreover, on several occasions certain specimens of blood from wild flies had a strong rank odour so suggestive of the "musky" odour of a crocodile which scents the whole creature, that it was difficult to believe the blood had not come from a crocodile. These specimens are numbered 5, 6, 7, and it will be seen that they are considerably larger than the one under discussion.

Altogether, 46 different specimens of blood have been measured, and they are arranged below in descending order of length, and in groups. The measurements represent the actual size in micro-millimetres.

¹ Twelve corpuscles only measured.

² Common brown species inhabiting holes in ground.

³ From a specimen kindly lent me by Miss Robertson.

⁴ Flies fed on a freshly killed specimen and blood from them taken as the type, one or two hours after feeding.

Table VIII.

Group I.

No.	Length in micromillimetres.	Breadth in micromillimetres.	Breadth-Length Ratio percentage.
1	18·2	8·9	49·1

This single specimen stands rather isolated. As regards length alone it might have come from a *tortoise*, though the breadth-length ratio is considerably below that type.

Group II.

No.	Length in micromillimetres.	Breadth in micromillimetres.	Breadth-Length Ratio percentage.
2	17·7	7·6	54·6
3 ¹	17·6	9·9	56·1
4	17·6	9·5	53·7
5 ²	17·3	9·3	53·3
6 ²	17·3	9·5	54·8
7 ²	17·2	9·6	55·8
8	17·	9·3	54·9
9	16·7	8·9	52·9
10 ³	16·7	7·8	47·
11	16·5	8·8	53·1
12	16·5	7·8	47·6
13	16·4	9·	54·9
14	16·3	7·9	48·7
15 ⁴	16·3	7·9	48·7
16	16·2	8·4	51·1
17 ⁵	16·2	8·2	50·7
18	16·2	8·4	51·7
19	16·1	8·4	52·

This group seems homogeneous, the members being closely connected by intermediates.

The last few, particularly 16-19, might be thought to have come from Varanus, but, as has been shown, no specimen from a fly should be ascribed to a type *smaller* than itself unless the difference is *very* minute.

As regards *length* only, all those below 8 might have come from a snake (python's blood measures 16·7 μ), but the breadth-length ratio is in no case so large as in a snake.

¹ Hæmogregarines present.

² Very strong crocodile odour.

³ 25 corpuscles only measured.

⁴ 31

⁵ 33 " " "

Similarly, though specimens below No. 4 might have come from a batrachian, they probably have not done so. I am inclined to ascribe this group to *crocodiles*.

Group III.

No.	Length in micromillimetres.	Breadth in micromillimetres.	Breadth-Length Ratio percentage.
20	15.9	8.3	52
21	15.8	8.3	52.6
<i>Varanus type</i> ...	15.7	8.6	50.9
22	15.7	7.9	50.5
23	15.5	8.3	53.7
24	15.5	8.1	52.5
25	15.4	8.3	54
26	15.4	7.9	51.7
27	15.3	8.9	57.3
28	15.3	8.8	57.7
29	15.3	8.1	53.2
30	15.3	8.4	54.8
31	15.2	8.4	55.6
32	15	8.3	55.1
33	15	7.5	49.6
34	15	8.3	55.3
35	15	8.3	55.3
36	14.7	7.4	50.6
37	14.5	7.9	54.3
<i>Brown Lizard</i> ...	14.4	7.2	50
38	14.4	8.4	58.2
39	14.3	7.8	52.3
40	14.1	8	56.8
41	14	7.4	52.7
42 ⁶	13.9	7.5	53.5
43	13.3	7	53.2
44	13	7.4	56.5

The members of Group III. may have been derived altogether from *Varanus*. But some of the lower members (particularly 44) had not the appearance of having shrunk so much as would have to be the case had they come from *Varanus*, and a smaller type must be found for them, which may possibly be some other lizard.

Group IV.

No.	Length in micromillimetres.	Breadth in micromillimetres.	Breadth-Length Ratio percentage.
<i>Cormorant.</i>			
a. Fly 1 hr. after feeding.	12.1	6.6	54.1
b. Fly 2 hrs. after feeding.	11.2	6.4	57.1
45	12.2	6.2	50.6
46	11.5	6	52

⁶ The fly contained numerous *Tryp. grayi*.

In two flies only has blood been found corresponding with that of a cormorant.

Assuming the above placings to be correct, the following table can be made out, giving the results at a glance.

Table IX.

Source.	Per-centage.	Actual No.	Source.	Per-centage.	Actual No.
Avian ...	4.3	2	Saurian Crocodile ? Tortoise	56.8	25
Reptilian ...	95.7	44		40.9	18
Amphibian ...	0.	0		2.3	1
	100.0	46	—	100.0	44

It thus appears that *reptilian blood is over twenty times more often found in wild flies than avian*; and that it is probably derived from saurians more often than from crocodiles.

Amphibian or ophidian blood has never been found. Forty-six flies were the material from which this result was derived, representing many hundreds of flies actually examined. For out of a batch of several hundred flies caught on one day those that had obviously fed recently were selected. On examination the majority were always found to have fed upon mammals, and of those that had fed on non-mammalian blood in only a few cases—sometimes in none—was the blood fresh enough to enable drawings of unaltered corpuscles to be made.

So that the table given above embodies the results of examination of many hundreds of flies and may be considered to be representative.

II. Vegetable Juices.

The question whether *Glossina* feeds from vegetable juices is a difficult one to answer definitely.

Observation in the field has been of no effect—the observer himself probably attracting the flies so that all chance of seeing one actually feeding from vegetable juices is lost. I have never seen *Glossina* feeding from flowers. The large heads of flowers of *Haronga madagascariensis*, Chois: (Hypericaceæ), which abounds on the island at the borders of the forest, are extremely attractive to insects, and I have seen a female *Tabanus fasciatus* feeding therefrom; but although *Glossinæ* were plentiful enough in those localities they were never seen on the flowers.

On one occasion I took a large slice of Papai fruit (which is attractive to mosquitoes) on to the fly beach and sat down by it but although *Glossinæ* often settled upon it none made any attempt to feed from it.

Since the Progress Report was sent in, the gut contents of 1,239 more flies have been examined with a view to obtaining definite evidence, 239 on Damba Island, 1,000 on Bugalla Island. Numerous bodies of apparently vegetable nature were found, some of which have been previously figured, others not hitherto met with are figured below. In all cases the fluid used to moisten the preparations was pure rainwater collected into a stoppered bottle

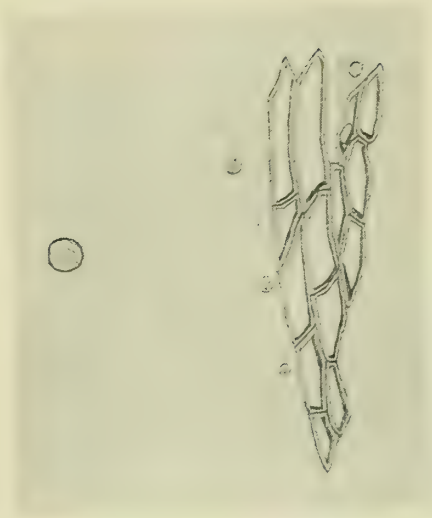


FIG. 19.

Piece of vegetable parenchyme with
chloroplasts.

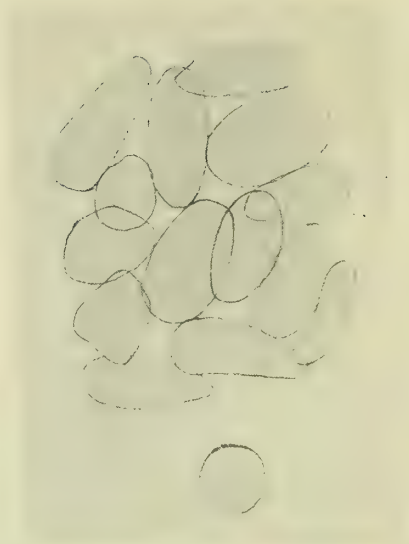


FIG. 20.

Banana starch grains.

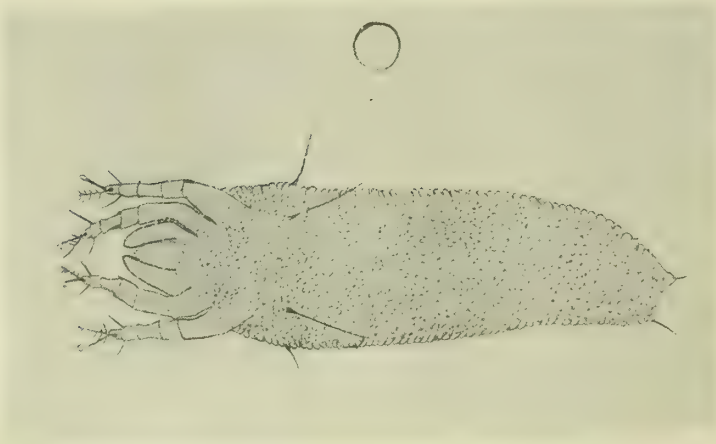


FIG. 21.

Acarine from proboscis.

In each drawing is a human erythrocyte on same scale.

from off the tent roof after this had been well washed in the heavy rain.

On several occasions undoubted pieces of vegetable parenchyma were seen, as for instance, Fig. 19.

On several occasions *Starch grains* have been found in preparations made from a fly's gut. They agreed in lines of growth, position of hilum, shape, size, &c., with *Banana starch*.

Now, on the first occasion on which I had observed these on Damba Island, a young pet monkey had been clambering about on my lap and shoulders, eating banana. Since the preparation might conceivably in some way have thus been fouled by small pieces dropped by the monkey, this first occasion was disregarded.

Subsequently, however, on two or three more occasions, on Bugalla Island where this source of error could be disregarded, similar starch grains were found (Fig. 20).

It is, however, difficult to understand how a fly could by suction dislodge a portion of the very adhesive mass of a banana. Perhaps the most definite evidence on this question is afforded by the following.

The probosces of the flies were examined in 638 cases, in all cases the proboscis being snipped off and examined by itself. In one case a living acarine of the family *Eriophyidae* or *Zemiostigmata* was found in the preparation; it seems probable that it had been clinging to the proboscis (Fig. 21).

The facts strongly suggest that the fly had plunged its proboscis into such a gall and that one of the acarines had clung to it.

III. Water.

Though prolonged observation in the field has not resulted in a fly being seen to imbibe either dew drops, or water from the lake or elsewhere, definite microscopical evidence has been obtained that they do so on occasion.

In one fly out of the 1,000 examined on Bugalla Island, an *Ostracod Crustacean* of minute size was found; it was not too large to have been sucked up the fly's proboscis (Fig. 22).

This has only been seen once in the examination of 600 flies at Jinja, 695 on Damba Island, and 1,000 on Bugalla Island.

Other objects, whose origin is uncertain, found in preparations from the gut of flies, are figured (Figs. 23-34).

I have never been able to find any of the Desmids which abound in species as well as in individuals in the sediment along the lake margin.

Conclusions re Food Supply.

I. The abundance of Speke's Tragelaph on the islands probably accounts for the great frequency of occurrence of mammalian blood in wild flies of that locality.

II. Birds are a relatively unimportant source of non-mammalian blood (4 per cent.); reptilian blood occurring in 96 per cent. of the flies which had fed on non-mammalian blood.

III. Varanus is probably of more importance than the crocodile; but other reptiles may also be fed upon.

IV. Glossina does suck up water.

V. Strong evidence has been obtained that it feeds from plant tissues.

Trypanosomes.

It is convenient next to proceed to the subject of Trypanosomes. The flies on Bugalla Island, Sesse, are still infected with a trypanosome, infective to a monkey, in whose blood they have all the appearance of *T. gambiense*; but no means were at hand of settling their identity by animal inoculations. Immediately I arrived at Bugalla, early in 1912, flies were put on a young ♂ monkey. Between January 18th and March 9th, 7,538 flies were put on it, and on March 16th it showed trypanosomes for the first time.

The monkey was examined about every week until the end of February, 1913, when I departed on leave, and it was liberated. At no time were trypanosomes at all numerous, and the monkey remained in the best of health and spirits.

Trypanosomes were last seen on December 31st, 1913, and the last examination on February 25th was negative.

The following table gives details of the experiment.

Table X.

Date.	♂ flies fed on monkey.	♀ flies fed on monkey.	Total.	Blood.
1912.				
January 18 ...	67	17	84	—
" 19 ...	128	36	164	—
" 22 ...	89	40	129	—
" 23 ...	126	24	150	—
" 24 ...	235	55	290	—
" 25 ...	266	68	334	—
" 27 ...	381	57	438	—
" 29 ...	330	35	365	—
" 30 ...	321	50	371	—
February 1 ...	430	40	470	—
" 2 ...	280	51	331	—
" 3 ...	433	80	510	—
" 4 ...	85	10	95	—
" 6 ...	490	101	591	—
" 8 ...	560	90	650	—
" 13 ...	500	100	600	—
" 15 ...	—	—	—	—
March 4 ...	180	60	240	—
" 5 ...	300	80	380	—
" 6 ...	280	51	331	—
" 7 ...	300	80	380	—
" 8 ...	215	60	275	—
" 9 ...	290	60	350	—
" 11 ...	250	56	306	—
" 12 ...	235	37	272	—
" 13 ...	375	93	468	—
" 14 ...	207	81	288	—
" 16 ...	420	90	510	+
			7,538 (up to March 9.)	

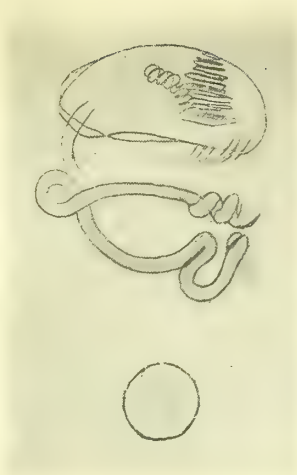


FIG. 22.

An Ostracod—The gut was extruded through pressure.

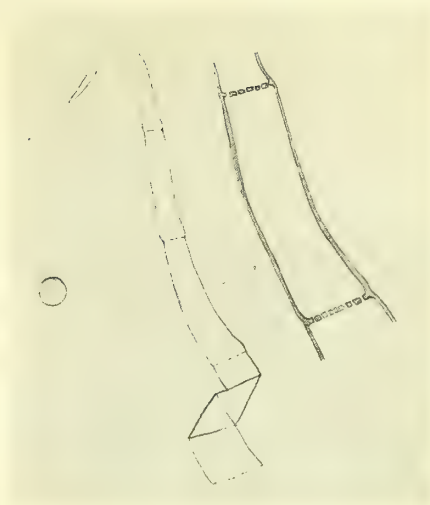


FIG. 23.

A piece of vegetable tissue; an enlarged drawing on the right.

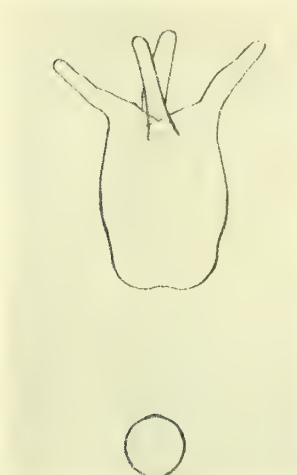


FIG. 24.

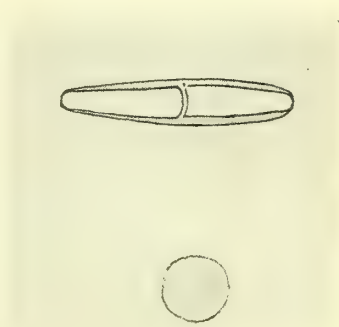


FIG. 25.



FIG. 26.

A translucent, faintly green, body without visible contents.
Human erythrocyte in each drawing on same scale.



FIG. 27.

Colourless translucent granular bodies.

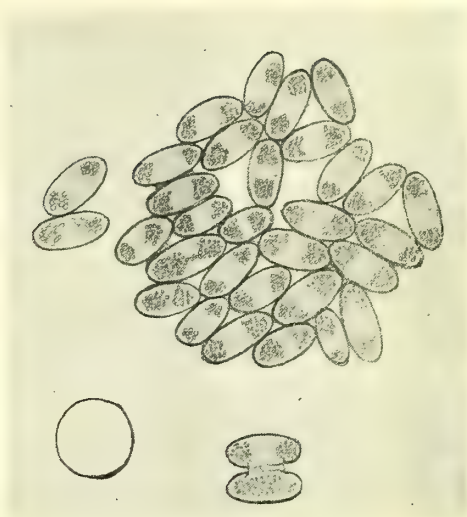


FIG. 28.

A very large number of these were found—they were dark in tint and slightly greenish.

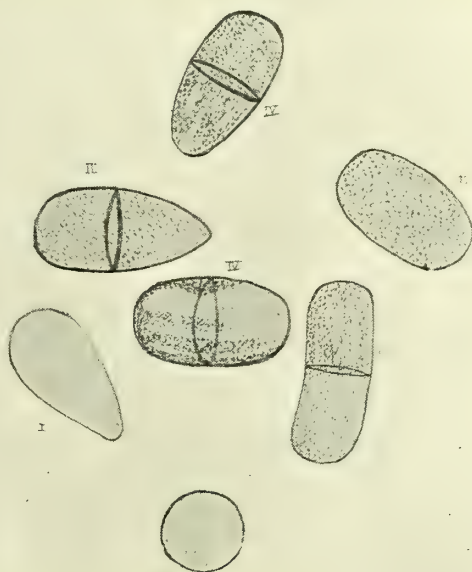


FIG. 29.

These bodies (Fig. 29) appear to be of the same nature as those in Fig. 28, but show further stages of development (i to iv). An equatorial girdle develops, and the granulation becomes arranged in bands.

A human erythrocyte in each drawing on the same scale.

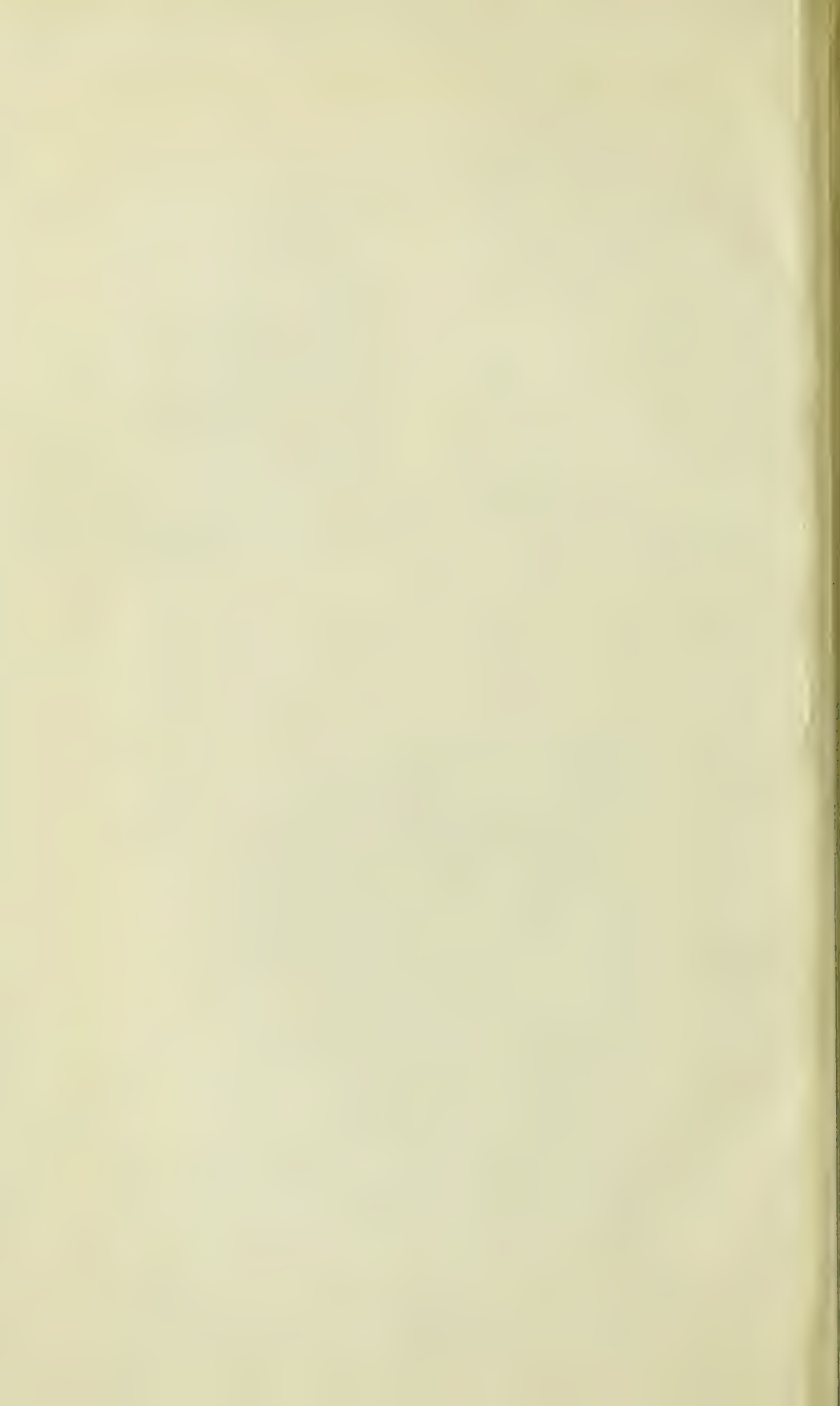




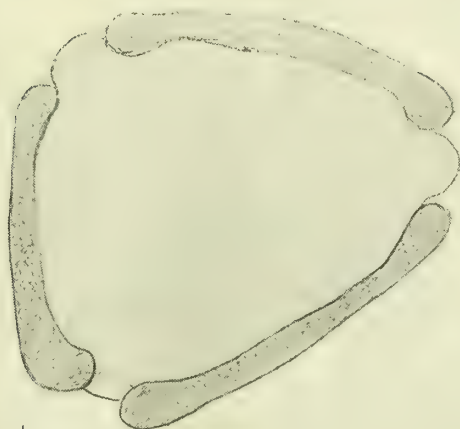
FIG. 30.

Numbers of these bodies in one fly. Only the surface plane is shown, they being composed of two or more layers. Dark green, with thick cell walls.



FIG. 31.

Large numbers of these in one fly, they may be a yeast.



In optical section.



Surface view.

FIG. 32.

Camera lucida drawing. The object is shown in optical section. It had thick walls, with an orifice at each angle to which an air bubble was attached. The surface appears full of irregular spaces, a small part is shown on the right.



TRYPANOSOMES AND CARMINE GRANULES.

It was mentioned in the Progress Report that there appears to be some association between the presence in the gut of wild flies of trypanosomes and amorphous chromatic granules which were alluded to as "Carmines granules."

This association is further borne out by the following Table XI., giving the results from Jinja on the mainland, Damba Island, and Bugalla Island. No distinction is drawn in this table between *Trypanosoma gambiense* and *T. grayi*.

Table XI.

Locality.	No. of flies examined.	Percentage containing Tryps. in gut.	Percentage with carmine granules in gut.
		Per cent.	Per cent.
Jinja	600	11	5.5
Damba	695	3.4	3.7
Bugalla	1,000	1.7	0.5

From this it appears that where the percentage of flies containing these granules is highest, there also is the highest percentage infected with trypanosomes, and *vice versa*.

It is worth noting that Jinja was the *first* locality at which I worked, so that it is possible that from inexperience the figures are *under* estimated. Even after considerable experience in examining wild flies, I found the percentage infected on Bugalla, together with that of the carmine granules, is much below that at Jinja, Damba being intermediate.

On some occasions the mass of granules was seen partly carmine and partly orange, individual pieces showing leading from one to the other. Trypanosomes have also been seen to be associated with these orange masses only, which appear to be merely a stage of the commoner carmine form.

In Damba ♂ fly 682 there were a few carmine granules in the hind-gut only, associated with a few trypanosomes—probably *T. grayi*.

In Sesse fly ♀ 912 there were granules in the gut just at the point of entrance of the malpighian tubules, and there were a few trypanosomes in the mid gut.

It is convenient to give here a summary of the facts ascertained with regard to these granules.

- I. In the locality where the granules were of most frequent occurrence in 51.5 per cent. cases in which they were found there were also trypanosomes, and in 30.3 per cent. cases in which trypanosomes were found there were also these granules.
- II. In $\frac{17}{2} = 80.9$ per cent. of the cases in which the granules were present in sufficient number to make the whole gut appear pink, trypanosomes were also found.
- III. In some cases, where part only of the gut was pink, trypanosomes were far more numerous in that part.

- IV. On Bugalla Island, where both trypanosomes and the granules were of least plentiful occurrence, the latter were never seen in sufficient quantity to colour the gut pink.
- V. Of three localities, that showing the highest percentage of flies infected with trypanosomes showed also the highest percentage of carmine granules present, and *vice versa*.
- VI. The granules may be found in the absence of trypanosomes, and *vice versa*.
- VII. The granules have been seen in a freshly-bred fly, and in a larva withdrawn from the body of a parent in which there were none.
- VIII. In one or two flies the granules were only present in the gut at the entrance of the malpighian tubes.
- IX. They may appear as orange, less sharply defined and less refractile masses which graduate into the typical sharply defined and bright carmine granules.

A possible explanation of all these facts is that the chromatic material is a visible expression of some metabolic peculiarity in the fly, which renders it peculiarly suitable for the development in it of trypanosomes should it happen to feed upon an infected animal.

Good proof or disproof of this theory could be afforded by noticing, in a batch of bred flies put to feed on an infected animal (of which only a small percentage will acquire infection), whether, of those that showed trypanosomes on dissection, a larger percentage contained these granules than did not contain them. The theory, however, involves the assumption that the number of flies showing this peculiarity of metabolism varies in different localities.

Conclusion.

Further evidence is given of some inter-relation between trypanosomes and the carmine granules sometimes found in the gut of flies.

TRYPANOSOMES AND BACILLI.

It was mentioned in the Progress Report that bacilli were only present in the gut of about 20 per cent. of flies. More careful examination, however, by dividing the gut into three parts, has shown that bacilli are practically always present in the forepart.

So that what has been said on the incompatibility of bacilli and trypanosomes requires modification: but, nevertheless, although they may occur together, it is quite exceptional to find more than a minimal number of bacilli in the foregut of a fly heavily infected with trypanosomes.

TRYPANOSOMES AND TRAGELAPHIS.

The fact that on Damba Island only 885 flies were needed to infect a monkey, whereas on Bugalla over seven thousand were required, is correlated with the fact that microscopical examination of wild flies on Damba showed trypanosomes to be twice as frequent as on Bugalla.

It was suggested earlier in this paper that the great preponderance of mammalian over non-mammalian blood in the gut of flies on Bugalla, was due to the unequalled facilities afforded to the fly for feeding from the tragelaph which rest throughout the day in the fly forest.

Putting this fact side by side with the small infectivity of the fly on Bugalla, as compared with Damba, it seems probable that the tragelaph on Bugalla are not, for some reason, highly infected with trypanosomes. This is borne out by the fact that Dr. H. L. Duke, who came to visit my camp for the express purpose of repeating the Damba results, was unable to infect experimental animals with *T. gambiense* by means of blood from the Bugalla tragelaphs, although more antelope were used than on Damba, where a positive result was obtained.

T. vivax.—Dr. Duke has, however, informed me by letter that he had infected a goat with *T. vivax* by inoculation of blood from a Bugalla tragelaph. I therefore subsequently examined the proboscides of a number of wild flies, and found in 14 out of 638 cases that the proboscis alone was infected with trypanosomes.

Conclusion.

So that it may be assumed that *T. vivax* was present in 2.2 per cent. of wild flies on Bugalla, thus exceeding *T. gambiense* in frequency of occurrence.

SEX PROPORTIONS.

The percentage proportion of ♀ ♀ in three localities was :—

Table XII.

Locality.	Time.	No. of flies on which result was based.	Female percentage.
Jinja (mainland) ...	July, 1910, to Feb., 1911	12,773	55.7*
Damba Island ...	March, 1911, to Dec., 1911	Over 6,000	21.6
Bugalla Island ...	Jan., 1912, to Feb., 1913	56,675	20.6

The monthly percentage of females, given in the charts previously figured (2 and 3) is seen to vary considerably, and it was suggested earlier that as the curve varied inversely as the temperature it seemed to indicate a greater susceptibility of the female to higher temperatures, so that at such times a greater proportion of females than males die. This is borne out by the above table, for Chart No. I., comparing the mainland (Entebbe) data with those of Bugalla, shows that the average temperature for each month of Bugalla is higher than that of Entebbe.

Whether the greater susceptibility of the female fly to higher temperatures is the *only* cause of the variation in sex proportion is another matter. I have been unable to throw any further light

* Given erroneously as 53 per cent. in Progress Report.

on the subject. The ratio in which the sexes are *produced* has no bearing on the question. The monthly curve, as plotted out in Chart IV., varies very greatly, and has no relation to the curve of the percentage of females amongst caught flies.

The pupæ from which the flies were bred were deposited during one month, and the flies that emerged from them are recorded under that month for comparison with the percentage of caught females (*see* Chart IV.). So that, if meteorological conditions have any influence in determining the sex of a larva, they would be, on the whole, those of the month previous to that under which the percentage of bred females is recorded, during which the larva was growing in utero and exposed to variations in meteorological conditions. Accordingly a chart was prepared in which the figures for the bred flies for each month were put back under the meteorological conditions for the preceding month, but from this no possible relationship, direct or inverse, could be made out between the curves of the percentage of bred females and the relative humidity.

The percentage of caught females, therefore, does not depend upon the number that are hatched out during that month.

It seemed possible that one reason why the *caught* female percentage is so much greater than the *bred* percentage, might be the removal of pupæ from their natural surroundings, exposed to variations in temperature, humidity, etc., to the laboratory where conditions are more uniform. For since the female fly appears to be more susceptible than the male to higher temperatures it might be supposed that the same would hold good with the pupæ, and that female pupæ die under natural conditions which are preserved by removal to the laboratory, so that the percentage of bred females is thus raised abnormally high.

Accordingly, the following experiments were made to test this. Of a batch of pupæ obtained at one time from the same place some were exposed to sun for a quarter of an hour, uncovered with earth, the others kept as a control.

Of another batch some were submersed for 48 hours, the rest kept as a control. The subjoined Table XIII. shows no appreciable difference in either case between control and experimental pupæ:—

—				No. of pupæ.	No. of flies emerging.	Percentage ♀ flies.
I.						Per cent.
Control	...	...	...	253	210	52.1
Immersed	...	...	...	200	125	52.8
II.						
Control	...	...	...	124	52	46.2
Exposed to sun	...	...	...	124	27	48.2

Conclusion.

The percentage of wild female flies seems to depend upon the effect of temperature upon the flies themselves.





FIG. 35.



FIG. 36.

Typical sites for collections of pupæ. Both only a few yards from edge of water and a few feet above it.

BREEDING GROUNDS.

I am quite convinced that a localised breeding ground is not by any means a necessity. When the conditions are not suitable on a large scale the flies deposit pupæ in any little nook that may offer the requisite shelter.

Thus, on a long stretch of shore on Damba Island when there was no loose, dry, sheltered soil to make a "breeding ground," my fly boys found a pupa at the base of a tussock of grass in quite a damp spot.

Nowhere amongst the several Sesse Islands that I have examined have I been able to find any localised breeding ground where pupæ could be found in numbers at all approaching those on Damba, where several thousand could be obtained monthly. The map of the locality on Bugalla Island, where I was settled, indicates stretches of shaded, sandy shore, where one might expect to find numbers of pupæ (*see* p. 2). Only one locality, however—that on the east side of the peninsula which was chosen as the "fly ground" (Fig. 1)—yielded any numbers, and that only as many hundreds as Damba yielded thousands. Yet flies were, if anything, more numerous on Bugalla Island than on Damba.

Possibly one explanation is that on Damba there was a long stretch of the bushes which were alluded to before as making admirable shelter for the pupæ (*Alchornea cordata*, Benth: *Euphorbiaceæ*); whereas on Bugalla the whole length of sand, several hundred yards, only provided two really suitable nooks—one under the shelter of a fallen trunk, the other under the overhanging butt of a tree amongst the roots (Fig. 35, and Fig. 36).

A little further south of this locality, in an adjoining bay, was a much smaller stretch of sandy beach, but there was not much shelter where the sand was soft and loose, and the few light bushes only yielded at their roots about 150 pupæ at one time. For very many miles south of this along the coast of Bugalla Island there was no place at all suitable.

On the north coast of the peninsula was a good strip of sandy shore, and here pupæ were searched for thoroughly, but only a few dozen scattered specimens could be found.

Westwards, along the whole shore of the huge bay, was no suitable place, the shore being either bare rock, or with rushes.

But just round the west point of the bay (and 9 or 10 miles in a direct line from my camp) was a tiny sandy sheltered cove, and here, under light bushes, a few dozen pupæ were collected.

Several of the larger islands of the Sesse archipelago (*e.g.*, Bufumira and Bunyama), the whole coast of which I have examined, going round them in a canoe, showed no spot suitable for a collection of pupæ.

Conclusion.

The prospects, therefore, of getting rid of Glossina by limiting one's attention to localised breeding grounds are not good. An attempt was made to ascertain whether female flies congregate from a distance at any one favoured spot for the purpose of larviposition.

On the shore of the bay opposite to the "fly ground" flies were caught for several days and marked. The boys then caught at the fly ground. It was found, however, that both males and females of the marked flies had come round, so that it was evident that the distance was not beyond the range of ordinary long-shore flight.

As regards localised breeding grounds, a useful indication of where to look for pupæ is often afforded by the pits of a species of "ant lion" (larvæ of the Myrmeleonid *Macroleon polyzonus* Gerst.). These insects require fine, dry loose soil or sand in which to make their pits, and where this occurs in shady localities close to water one finds the pits together with *Glossina* pupæ.

It is worth noting that a live *Glossina* pupa from which the fly has not emerged is very much harder to see than the empty shell. The skin of the larva when hardening becomes coated with very fine particles of sand or earth, which adhere firmly to the pupal case, making it very difficult to be distinguished amongst the fine earth wherein it lies. This may account for the fact that when one first begins to search for pupæ one finds only the smooth, conspicuous empty cases. It took me a long time before I learnt to find the live pupæ themselves.

Summary of Conclusions.

- I. A male fly has been re-caught 247-253 days after it was marked; a female fly 182 days after it had been marked.
- II. Climatic conditions affect *Glossina fuscipes (palpalis)* in three ways:—
 - (a) Decreased relative humidity is unfavourable to the adult fly.
 - (b) Higher temperatures are particularly unfavourable to the female sex.
 - (c) There is an inverse relation between relative humidity and rate of larviposition.
- III. In addition to those enemies recorded in the Progress Report, the following have been observed to attack *Glossina*:—
 - (a) Larva. An ant; species not known.
 - (b) Newly emerged fly. An Asilid fly; species not known.
 - (c) Adult fly. An Asilid; species not known. The dragon fly, *Orthetrum farinosum*.
- IV. No evidence has been obtained that birds devour *Glossina* in any stage; nor is it probable that they do so.
- V. Sundry Acarines have been found, which were probably external parasites.
- VI. Nematodes have been found in the abdominal cavity of four out of over a thousand flies.
- VII. The abundance of Speke's Tragelaph on the islands probably accounts for the great frequency of occurrence of mammalian blood in wild flies of that locality.
- VIII. Birds are a relatively unimportant source of food—their blood only occurring in four per cent. of the flies which had fed on non-mammalian blood: 96 per cent. being reptilian.

- IX. *Varanus* is probably of more importance than the crocodile, but other reptiles may be fed upon.
- X. *Glossina* does suck up water.
- XI. Strong evidence has been obtained that it feeds on plant tissues.
- XII. Further evidence is given of some inter-relation between trypanosomes and the carmine granules sometimes found in the gut of flies.
- XIII. In a single experiment on Bugalla Island over 7,000 flies were needed to infect a monkey with a trypanosome having the appearance of *T. gambiense*.
- XIV. *T. vivax* was found in the proboscis of 2.2 per cent. of wild flies on Bugalla Island.
- XV. The percentage of wild female flies seems to depend upon the effect of temperature upon the flies themselves.
- XVI. The prospects of getting rid of *Glossina* by limiting one's attentions to localised breeding grounds are not good.

It may be useful to bring together a few scattered notes on the habits of the fly.

The time when they are most eager to feed is early in a morning after a little rain, when the sun is hardly through the clouds, and it is close and still. On such days it is impossible to avoid being bitten; the flies will follow one with utmost persistency, and are readily carried some distance on one's clothes. Conversely, the time to be in the fly area if one wishes to avoid being bitten is in the middle of a day on which there is a fresh breeze, cloudless sky, and brilliant sun.

On such days, though there may be numbers of flies about, and they will readily come and sit on one's clothing, they are not eager to feed.

In the early afternoon the flies are not at all active until about 4 p.m., when they again become eager to feed till dusk. As has been said, this was found to be the time on Bugalla at which the *Tragelaph* come out and feed, and are freely exposed to the attacks of flies.

SOME TRYPANOSOMES RECOVERED FROM WILD GAME IN WESTERN UGANDA.

H. L. DUKE, B.A., M.B., B.C., D.T.M. & H., CAMB.

The tour of investigation dealt with in the following pages, was undertaken with the object of obtaining information regarding the part played in wild game in the spread of trypanosomiasis of human beings and domestic animals. The region traversed, the Western Province of the Uganda Protectorate, is a great game country with a relatively small population.

The route followed is indicated on the accompanying Map as a dotted red line, and the main data available regarding the distribution of the various *Glossinæ* are also given. Dr. van Someren's report of his investigations in this district as to the distribution of "fly" and of Sleeping Sickness enabled me to select without trouble the most favourable sites for experiment, and I wish to express my admiration for the immense amount of trouble and accurate observation he expended in its production. A similar report dealing with the Semiliki Valley by Dr. McConnell was also referred to.

The bulk of the experiments given in this paper were performed in areas where tsetse were more or less abundant. The places visited are given on the map, together with some information regarding fly distribution compiled by Dr. van Someren. As a control to the inoculations other experiments are given which were carried out in Budu and other tsetse-free districts, including some unpublished results obtained in March, 1912.

The tsetse regions traversed may be roughly divided into four districts, each with its characteristic topography and predominant game species. These will now be briefly considered in the order visited.

District No. 1.—The north-west corner of Ankole Province, as defined by the north-east shore of Lake Edward, the Kazinga Channel and the south-west corner of Lake George.

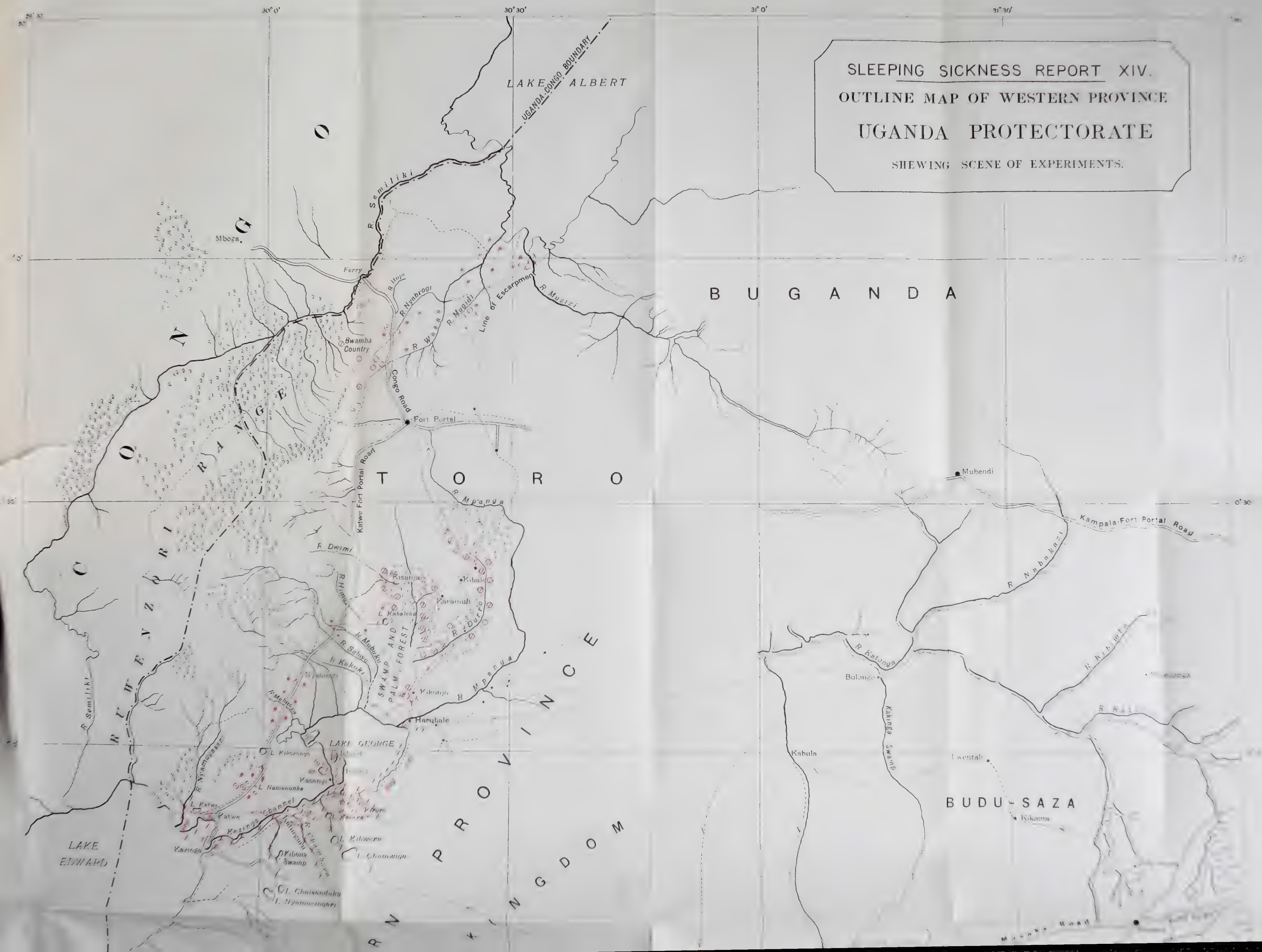
The country consists of a flat, grassy plain along the shore of Lake Edward, extending eastwards to the foot of a steep escarpment, some 1,700 feet in height, which as it approaches Lake George breaks up into a succession of hills and valleys among which lie the picturesque Crater Lakes.

Several small rivers, their banks forest-clad and fly-ridden, run down from the hills into the Kazinga Channel or Lake George, and other streams lead into or out of many of the Crater Lakes. The whole country is covered with coarse grass and scattered thorn trees, with numerous thickets and occasional small patches of scrub.

In the neighbourhood of Lake Kiraro and towards the southern end of Lake George the thorn trees are often so closely placed as to almost merit the title of forest. The mouths of the larger streams are blocked with masses of papyrus, which is also found to a varying extent fringing the Crater Lakes and all around Lake George.

Lake Kiraro is surrounded by a belt of forest and *G. palpalis* is found in considerable numbers. A papyrus swamp marks its outlet into Lake George. The population of this district is confined to one or two scattered collections of huts belonging to fisher folk along the shore of the channel and of Lake George. Van Someren reported Sleeping Sickness among the natives in 1910, and I was able to confirm his opinion that *G. palpalis* is the only tsetse present. The game is numerous and varied. Waterbuck occur in great numbers, especially among the hills about the Crater Lakes. Buffalo frequent both the Crater Lakes and the shore of Lake George, but not in great numbers. They

SLEEPING SICKNESS REPORT XIV.
OUTLINE MAP OF WESTERN PROVINCE
UGANDA PROTECTORATE
SHEWING SCENE OF EXPERIMENTS.



WESTERN KOKOLE

BUKANGA

REFERENCE

- Sleeping Sickness
- Glossina Palpalis XXX
- .. Morsitans O
- .. Pallidipes //
- .. Fusca Brevipalpis O
- Route followed - - - - -
- High Roads = = = = =

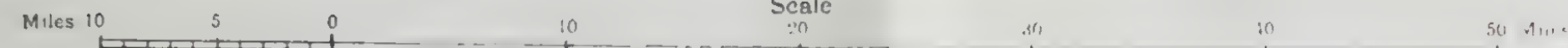
30° 0'

30 30'

Scale

31° 0'

31° 30'



Printed at the Ordnance Survey Office, Southampton, 1923

are said to be very numerous on certain islands in the south-west corner of the lake. Elephant are also occasional visitors. On the plains Uganda cob and reedbuck are very plentiful. Bushbuck, bushpig, warthog, duiker and hippo are also found, the latter being especially numerous in Kiraro Lake. It will be seen later that a very interesting trypanosome was obtained by feeding wild *G. palpalis*, caught at Lake Kiraro, on a monkey. This lake is surrounded by forest and is very rarely, if at all, visited by natives from the huts on the surrounding shores of Lake George. It swarms with hippo, which make it impossible for natives to fish, even were there any special inducement for them to do so. There are also no signs of native paths around the lake shore, and the fear of buffalo and hippo acts as a strong deterrent to visitors. I was unfortunately unable to obtain any of the species of game whose tracks proved them to frequent this tract, *i.e.*, buffalo, hippo and bushbuck. Two hippo were shot but not recovered until too late to inoculate their blood. Much stress cannot be laid upon the numbers obtained in a single fly experiment even though those numbers be small, but general considerations make it improbable that natives reach the Kiraro tsetse while wild animals, especially hippo, are exceedingly numerous all around the lake. From the distribution of the fly on this lake it is obvious that they are particularly numerous at the hippo landing places, and the exceptional number of these animals constitutes a never failing source of food.

As regards the smaller game which frequent the more open parts of the district, it is improbable that they are much exposed to the bite of *G. palpalis*. Thus the cob and reedbuck will play but a minor part in the spread of trypanosomiasis by tsetse in this region. Hippo, buffalo, elephant and bushbuck will by virtue of their habits be frequently exposed to the fly.

District No. 2.—The parts of Toro Province from the northern bank of the Kazinga Channel to the neighbourhood of Mahokya and the Hima river. This district consists of open plains, stretching up to the Ruwenzori foot-hills on the west and north-west, and giving place to thorn scrub and scattered thickets around Mahokya and Hima. To the east lie Lakes George and Kabaleka and the western forests of the next district. At the Hima river the open country comes to an end, and from thence northwards to the Semiliki escarpment elephant grass covers every open space.

The game includes buffalo, elephant, waterbuck, reedbuck and Uganda cob. Buffalo are found in great numbers especially in the district around Hima and Mahokya. *G. pallidipes* may be said to be the tsetse characteristic of this district. *G. palpalis* and *G. morsitans* also occur.

Dr. van Someren in 1910 reported Sleeping Sickness at Kasenyi Lake, and also on the shore of Lake George in the neighbourhood of Mahokya.

The distribution of *Glossina pallidipes* throughout the northern part of the district ensures the frequent exposure of all the game to fly bite. Van Someren found *G. morsitans* in this neighbourhood in 1910, particularly in the proximity of buffalo,

and these two *Glossina* will doubtless come into frequent contact with the smaller game whose habits do not lead them into *palpalis* areas. There are a considerable number of cattle in the district, among which van Someren found a disease due to *T. nanum* (cf. Proc. R.S., B, Vol. 85, 1912). The natives have long realised the danger of taking cattle into the immediate neighbourhood of Mahokya, and when transport is necessary are accustomed to drive them through during the night.

District No. 3.—The southern Toro Game Reserve, comprising the country round the northern end of Lake George, and running northwards nearly to Fort Portal.

This is a very sparsely populated district, including the great forests around the Duro river and its branches, the papyrus swamps and palm forests around Lakes George and Kabaleka, and a succession of grass-clad hills and narrow forested valleys, with here and there a single hut or a tiny village. The fauna include elephant, buffalo, giant and bushpig, warthog, hippo, eland. Colobus monkeys are found in the forests. *Glossina fusca* is found throughout the forests, and *G. pallidipes* and *palpalis* also occur. Dr. van Someren reported Sleeping Sickness in 1910 in a small village, Harubale, near the shore of Lake George, and since then the remaining natives are said to have all died of the disease. In the immediate vicinity of this village *G. palpalis* and hippo are very numerous, but no antelope were observed. Owing to the predominance of *G. fusca* among the *Glossina* of this district it was necessary when investigating the game reservoir to obtain forest-loving animals, to the exclusion of reed and waterbuck, which do not enter the big forests. Elephant, buffalo, hippo, bushbuck, giant and bushpig all frequent the *fusca* forests, but are all extremely difficult to obtain. The nature of the country and the very scanty population made it impossible to organise a drive, and the only animals brought in by natives were two young giant pig. It was therefore impossible to accumulate sufficient data on the infectivity of the game in this region to justify an opinion concerning *G. fusca* as a trypanosome carrier.

District No. 4.—The Semiliki valley north of the Mboga road. A flat plain, through which runs the Semiliki river, bounded on the west and east by the Congo and Toro hills respectively. To the north lies Lake Albert Edward. Towards the Toro escarpment the bare plain gives place to large thorn trees and Euphorbia thickets, which merge into a densely forested area around the opening of the Muzizi river into Lake Albert.

Game is very plentiful, including waterbuck, elephant, buffalo, cob, reedbuck, warthog, hartebeest and baboons. *G. pallidipes* and *G. palpalis* are common along the banks of the streams to the east of the Semiliki river. *G. fusca* is found in small numbers in the forest around the Mugidi river. No Sleeping Sickness has been reported from this region. The cob and reedbuck on the plain along the Semiliki river will probably never meet tsetse. On the eastern side of the valley, however, the game must be constantly exposed to *G. pallidipes* and less

frequently to *G. palpalis* and *fusca*. Cattle are confined to the fly-free western part of the valley, and appear to be free from trypanosomiasis. It is of interest to note that a dog belonging to the Provincial Commissioner which accompanied him on a visit to the south-eastern corner of Lake Albert in 1912 contracted trypanosomiasis and died. It must have been infected close to the lake, within a mile or two of the place where the hyaena of goat was shot, as tsetse are not found above the escarpment. The trypanosome proved to belong morphologically to the *T. pecorum* group; sub-inoculations were not made.

Another series of wild animals was shot in districts where no tsetse are known to occur. These include parts of Budu around Masaka and certain parts of Bukanga and Koki to the south of the Mbarara-Masaka road.

The object of these experiments was to serve as a control to the game in tsetse districts. Two of the elephants scheduled as shot in District 3 were actually obtained in a tsetse-free district, but the natives are practically unanimous in stating that these herds roam to and from the tsetse region of the southern Toro Game Reserve.

METHODS OF EXPERIMENT.

The blood from the freshly killed animal was placed in a tube provided with beads and immediately defibrinated. At the same time two slides were taken for subsequent staining and examination. Five c.c. were then inoculated into an experimental animal within four hours of death. At the time of inoculation two fresh preparations of the defibrinated blood were examined and two films were also taken for subsequent staining, each of which received from 20-30 minutes careful examination. In the earlier experiments the blood was received into a stock citrate solution, but owing to the difficulty of obtaining good water and the unsatisfactory behaviour of the rough scales of the safari outfit it was found impossible to renew the solution. In order to include as many species of trypanosomes as possible goats were used for the blood inoculation. This ensures the detection of such species as *T. nanum*, *vivax* and *uniforme*, which are not pathogenic to dogs and monkeys. To avoid fallacy all the experimental goats were purchased in tsetse-free districts and where there was no suspicion of any local cattle diseases. From the date of purchase they were examined daily and in no single instance was there any suggestion of a spontaneous infection apart from the inoculations. In dealing with the actual inoculation experiments, full data are given regarding the period of preliminary examination of each goat. From experience acquired during routine daily examination of a large number of goats at Mpumu I consider that the following is a good test as to whether first observed appearance of trypanosomes in these animals testifies to an old or fresh infection. If the parasites continue in the blood for several consecutive days after their first appearance and in considerable numbers then the infection is a fresh one. If, however, a single organism or very few are

found, which disappear on the following day, then there are strong grounds for suspecting the animals concerned of an old infection. Old trypanosome infections in goats are notoriously difficult to demonstrate microscopically, and the animal may become tolerant to the extent of keeping in good condition and only very occasionally showing trypanosomes in the course of the daily examinations.

Kinghorn and Yorke point out that they were unable to utilise goats for their experiments owing to the impossibility of obtaining "clean" animals. They quote, as an instance of the dangers of this source of error, a goat which only showed trypanosomes after being examined at frequent intervals for two months (cf. *Ann. Trop. Med. Liv.*, Vol. VI., 3, A., 1912). It is under just such circumstances that a chronic *trypanosomiasis* will escape detection. The examination must be daily otherwise the organism may escape detection during ordinary routine examination. As will be seen, all goats used for these experiments were examined daily, and on several occasions twice daily. Furthermore, in each case at the first appearance of trypanosomes in their blood the organisms were always present in considerable numbers, and for several consecutive days. In all instances also the trypanosomes have been observed more or less regularly during subsequent routine examinations at the laboratory, and several have shown signs of disease. As soon as trypanosomes appeared in the goats the animals were despatched direct to the laboratory. After the death of goat B from mishandling and neglect on the road to Kampala the animals were transported in light crates. The porters followed the main road to Mubendi, whence the goats were taken by motor car to Kampala. No tsetse exist along the route.

It is to be regretted that it was not possible to devote a separate goat to each wild animal shot. The continual moving from place to place, often by long marches under trying conditions, necessarily limited the number of experiment animals, to say nothing of the increased risk of fallacy. At the beginning of the tour an attempt was made to set aside a goat for each species of game, and so discover the relative infectivity of the different species. As time went on, however, this was found impossible owing to the great strain thrown on certain goats in certain districts. Nevertheless, as will be seen, a certain amount of grouping was maintained throughout the experiments, resulting in a "buffalo goat," a "waterbuck goat," and so on. The main object of the trip was to ascertain whether the game in the Western Province of the Uganda Protectorate harboured trypanosomes, and if so, of what nature. This object has been fulfilled as regards the main issues, though the actual percentage of infected animals cannot be determined. In the account of the individual experiments the full history of each goat is given, together with all blood inoculations which it received. As will be seen, many of the animals inoculated may be excluded as the source of the trypanosomes owing to the length of time the goat remained negative after their inoculation. On the other hand, several animals may demand consideration, and even if

microscopical examination of the blood of one of them be positive it is still impossible to arrive at a definite decision.

Kinghorn and Yorke noted on several occasions that trypanosomes found in slides of game blood did not necessarily appear in the subinoculated animals and the converse was also observed. As will be seen below trypanosomes were found in slides from two wild animals whose blood failed to infect the corresponding goats. Again only on two occasions were trypanosomes found in slides of the animals selected as the most probable origin of the trypanosomes in the goats.

Throughout the safari the strictest precautions were taken in selecting camps and routes to eliminate chances of infection by the bites of wild tsetse. My sincere thanks are due to Miss Robertson, who most kindly received all the goats on their arrival in Kampala and started the subinoculation experiments pending my return. Miss Robertson also kindly undertook the preparation of all figures.

The actual inoculation experiments will now be considered and an attempt made to diagnose the various trypanosomes obtained. Each goat will be dealt with in turn, the game inoculation being given first followed by subinoculation experiments, and an account of the morphology of the organism.

Goat A.—Bought in fly-free and healthy district. Negative to daily examination for 51 days prior to the first appearance of trypanosomes in the blood on January 10th, 1913.

Animal inoculated.	Date of inoculation.	District wherein shot.	Examination of inoculated blood.	
			2 fresh slides.	2 stained slides.
Buffalo ...	2.1.13	Fly II.	—	—
" ...	4.1.13	"	+	+

Trypanosomes appeared in the goat on the 6th day after inoculation of the positive buffalo blood. The following subinoculation experiments were carried out.

Animal.	Source of virus.	Incubation Period.	Duration of disease.	Remarks.
Goat 4 ...	Goat A.	8 days	53 days	Paralysis of hind quarters preceded death.
Dog 6 ...	"	10 "	56 "	Showed <i>T. pecorum</i> , and the organism figured in group 1, Nos. 34-35.
Dog 11 ...	"	8 "	18 "	Showed <i>T. pecorum</i> .
Rat 4 ...	"	15 "	23 "	
Monkey 11 ...	"	—	—	Did not develop trypanosomiasis.
Bullock 4 ...	"	9 ,	—	Shows <i>T. pecorum</i> , obvious symptoms noticeable.

Morphology of the trypanosomes.

Examination of trypanosomes taken from the subinoculated animals revealed the presence of three different species in the blood of Goat A.

(1) *T. uniforme*.—As figured in Plate 1, Group 1, Nos. 1-8. This organism shows the characteristic *uniforme* structure with a relatively large kinetic nucleus often jammed up against the posterior end of the organism. This trypanosome was seen in the blood of Goat A. It was not identified in Goat 4, which, however, showed comparatively few trypanosomes and did not receive much attention microscopically after the first appearance of the parasites in its blood.

(2) *T. pecorum*.—Seen in both the dogs, the rat, and the bullock of the above table; c.f. Group 1, Nos. 15-32.

(3) The species figured in Group 1, Nos. 33-35; of these the two last are obviously not *T. uniforme*, as the organism is not pathogenic to dogs. The size and general appearance of No. 35 also precludes this trypanosome. As will be seen it is possible that the organism corresponds with a type found in the original buffalo.

The trypanosomes found in the blood of the buffalo inoculated into Goat A on January 4, 1913.

(1) A long trypanosome figured in Group 1, Nos. 36-45 resembling *T. vivax* in structure. It reaches 31μ in length. When seen in fresh preparations of the defibrinated buffalo blood it did not show the characteristic *vivax* movements, the note being "a long apparently free-flagellated trypanosome with no translatory movement; very active wriggling in one place: clears a hole for itself among the corpuscles." This trypanosome has not been identified in any of the subinoculation experiments. It may be identical with *T. caprae*.

(2) A trypanosome differing somewhat in morphology from the one just described figured in Group 1, Nos. 34-35.

(3) The specimen figured in Group 1, No. 48. This trypanosome might belong to the same species as No. 35, or on the other hand it might be *T. pecorum*.

(4) A small trypanosome figured in Group 1, No. 49 which might be either *T. uniforme* or *T. pecorum*. It may be noted here that as the result of a large number of observations on *T. pecorum* that it is by no means uncommon to find this trypanosome bearing a short free portion to its flagellum. This may be seen in some of the figures of dividing examples given in Bruce's original paper (c.f. Proc. Roy. Soc.).

It is unfortunate that trypanosomes were somewhat scarce in the slides of the buffalo blood, the majority being of the long *vivax*-like type. The results obtained are however very interesting if only as constituting the first occasion on which

T. pecorum has been derived from wild buffalo. It is possible that the first of the two buffalo inoculated may have accounted for some of the organisms in Goat A, although nothing was seen in the blood. Figures 48 and 49 are, however, strongly suggestive of the *T. uniforme* and *T. pecorum* which developed in the subinoculated animals.

With regard to the nature of the trypanosome No. 33 and 35, nothing definite can be said as the only monkey inoculated from Goat A failed to take. Fig. 35 is, however, very suggestive of some *T. gambiense*-like organism of which Nos. 46 and 47 in the buffalo may be the origin.

Goat A itself has during the last fortnight shown signs of general debility without, however, any striking symptoms. Both the buffalo appeared in excellent condition when shot. The one inoculated on January 4, 1913, was a young adult cow, the other an old bull.

Goat B.—This animal became positive after receiving the blood of three waterbuck shot in the vicinity of Mahokya in fly-district 2. No parasites were seen in the blood slides of these buck. The goat unfortunately died while being driven to Kampala, without doubt, owing to the carelessness of the porters. In consequence of this accident all subsequent goats were despatched in crates.

Goat C.—Bought in fly-free and healthy district. Negative to daily blood examination throughout the tour and up to the date of writing, May 1, 1913. This goat thus serves to a certain extent as a control to its less fortunate companions.

Animal inoculated.	Date of inoculation.	District wherein shot.	Examination of inoculated blood.	
			2 fresh slides.	2 stained slides.
Waterbuck 1	16.12.12	Fly 1	—	—
" 2	16.12.12	"	—	—
" 3	17.12.12	"	—	+ (cf. infra).
" 4	18.12.12	"	—	—
" 5	19.12.12	"	—	—
" 6	21.12.12	"	—	—
" 7	22.12.12	"	—	—
Elephant ...	11.12.13	" 4	—	—
Hartebeest ...	18.2.13	"	—	—
Elephant 2 ...	11.3.13	" 3	—	—
" 3 ...	16.3.13	"	—	—

The failure to find trypanosomes on examination of the fresh blood preparations of Waterbuck 3 was probably due to a partial laking of the blood caused by faulty citrate solution. The stock supply had given out, and the freshly made solution which was employed on the 16th and 17th of December was discarded in favour of defibrinisation of the blood.

Morphology of the trypanosome found in the waterbuck.

The organism is figured in Plate 2, Group 3, Nos. 1-13. It will be seen as a small trypanosome provided with a free flagellum and having resemblances to *T. uniforme*. No. 1 has no free flagellum but is quite compatible with the *T. uniforme* described by Fraser and myself in 1911 (c.f. SS., Report No. 12). No. 13 measures some 23μ in length and is obviously a different species of organism to the others figured. Its structure suggests *T. vivax* which very commonly occurs together with *T. uniforme* in the *G. palpalis* caught on Lake Victoria.

Goat D.—Bought in fly-free and healthy district. Negative to daily blood examination for 17 days previous to the first appearance of trypanosomes in its blood on December 31, 1912.

Animal inoculated.	Date of inoculation.	District wherein shot.	Examination of inoculated blood.	
			2 fresh slides.	2 stained.
Uganda cob ...	17.12.12	Fly I.	—	—
Bushbuck ...	17.12.12	"	—	—
" ...	20.12.12	"	—	—
Uganda cob ...	20.12.12	"	—	—
" ...	22.12.12	"	—	—

No trypanosomes were seen in the blood of these antelope.

The following subinoculations were performed:—

Animal.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
Goat 2 ...	Goat D	7 days	—	Showed <i>T. nanum</i> .
Dog 5 (a) ...	"	—	—	Did not become infected.
" 2 ...	"	—	—	" "
Rat 18 ...	"	—	—	" "
" 19 ...	"	—	—	" "
Monkey 9 ...	"	—	—	" "

The trypanosomes of this Goat are therefore non-inoculable into dogs, rats, and monkeys, *i.e.* belonging to the *nanum-vivax-uniforme* group.

Morphology.

Cf. Figures, Plate 2, Group 4. Nos. 1-3 correspond to *T. nanum*. Nos. 4-7 have a marked free flagellum and suggest *T. uniforme*. The flagellum of these specimens excludes *T. nanum*. No. 9 measures 20μ in length, which is very large

for *T. nanum*, and No. 8 is apparently of the same type though smaller. More cannot be said with regard to this form, only two specimens of which were seen, one in Goat D and one in Goat 2. This Goat shows very marked signs of weakness and paralysis of the limbs. For the last three weeks it has been unable to walk more than a few steps without falling.

Goat E.—Bought in a fly-free and healthy district. Negative to daily blood examination for 31 days previous to the first appearance of trypanosomes in its blood on January 16, 1913.

Animal inoculated.	Date of inoculation.	District wherein shot.	Examination of inoculated blood.	
			2 fresh slides.	2 stained.
Bushbuck ...	1.1.13	Fly II.	—	—
Reedbuck ...	1.1.13	"	—	—
" ...	3.1.13	"	—	—
" ...	3.1.13	"	—	—
" ...	9.1.13	"	—	—

No trypanosomes were seen in the blood of these animals. Slides of antelope blood sent to Mpumu laboratory from this district by Dr. van Someren in 1910 were found to contain *T. uniforme* and *T. ingens*. The following are subinoculations made from Goat E.

Animal.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
Goat 6 ...	Goat E	17 days	67 days	Shown lumbar paralysis for two days before death. Shown <i>T. nanum</i> and <i>uniforme</i> .
Dog 5 ...	"	9 "	28 "	Both corneae became opaque; showed long trypanosome.
Rat 6 ...	"	9 "	29 "	Shows long trypanosome.
Monkey 3 ...	"	12 "	—	" "
Goat 13 ...	Monkey 3	9 "	—	" "
Guinea-pig 3...	Rat 6	4 "	11 days	" "

It is interesting to note that the blood of Goat E having been passed through monkey 3 no longer infects Goat 13 with the short organism.

Morphology.

Three species of trypanosomes are recognisable morphologically.

1. A large organism with well marked free flagellum, pathogenic to dogs and rats.

The following table gives measurements of one hundred individuals of this organism on a slide taken from the blood of Dog 5.

Animal.	16 μ	17 μ	18 μ	19 μ	20 μ	21 μ	22 μ	23 μ	24 μ	25 μ	26 μ	27 μ	28 μ	29 μ	30 μ	Average.
Dog 5 ...	1	2	—	4	8	6	11	11	14	9	9	16	5	1	3	23.93

This trypanosome is figured on Plate 3, Group 5, Nos. 12-24. The predominance of long and short forms in the blood varies on different days as with other polymorphic trypanosomes.

The undulating membrane is well marked. The kinetic nucleus is situated at the extreme end of the body or at a little distance from it.

A single form figured in Group 5, No. 17, shows a tendency to a posterior arrangement of the trophonucleus.

This trypanosome is comparatively rare in the blood of the goats examined. Nos. 10 and 11 are suggestive of the intermediate forms and were drawn from Goat 5. The kinetic nucleus of these examples is not that of *T. uniforme*.

Nos. 16, 17, and 19 of Group 5 show the blue line which occurs fairly frequently in the blood of both monkey and guinea-pig. This line was also seen in the fly strain discussed below. This trypanosome belongs clearly to the *brucei-gambiense-pecaudi* group.

2. A short trypanosome, non-pathogenic to rats, dogs, and monkeys. This organism is apparently *T. nanum* and is figured in Group 5, Nos. 1-5.

3. A *T. uniforme*-like trypanosome with free flagellum figured in Group 5, Nos. 6-9. The characteristic kinetic nucleus is very noticeable.

Goat F.—Bought in fly-free and healthy district. Negative for 68 days before the first appearance of trypanosomes in its blood on 2nd March, 1913.

Animal inoculated.	Date of inoculation.	District wherein shot.	Examination of inoculated blood.	
			2 fresh slides.	2 stained.
Buffalo ...	5.1.13	Fly II.	—	—
" ...	8.1.13	" II.	—	—
" ...	13.1.13	" II.	—	—
" ...	13.1.13	" II.	—	—
" ...	21.1.13	" III.	—	—
" ...	22.1.13	" III.	—	—
" ...	10.2.13	" IV.	—	—
Wart Hog ...	11.2.13	" IV.	—	—
Buffalo ...	12.1.13	" IV.	—	—
" ...	12.1.13	" IV.	—	—

No trypanosomes were seen in any of the above animals' blood. It will be noticed that the incubation period is 18 days, supposing one of the two last buffalo of the table to be responsible for the infection.

Subinoculations:—

Animal.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
Dog 9 ...	Goat F.	—	—	Did not become infected
" 16 ...	"	—	—	" "
Rat 13 ...	"	—	—	" "
" 14 ...	"	—	—	" "
Monkey 10 ...	"	—	—	" "

The organisms present in Goat F must therefore belong to the *nanum-vivax-uniforme* group of trypanosomes.

Morphology.

The trypanosomes from this goat are shown on Plate 4, Group 8, Nos. 1-13. Nos. 6 and 11 have a well marked free flagellum and suggest *T. uniforme*. The remainder are probably *T. nanum*.

Goat F shows no obvious symptoms.

Goat G.—Bought in a fly-free and healthy neighbourhood. Negative to daily blood examination for 53 days before the first appearance of trypanosome in its blood on 8th January, 1913.

Animal inoculated.	Date of inoculation.	District wherein shot.	Examination of inoculated blood.	
			2 fresh slides.	2 stained.
Eland ...	21.1.13	Fly III.	—	—
Reedbuck ...	10.2.13	" IV.	—	—
" ...	10.2.13	" IV.	—	—
Bushbuck ...	12.2.13	" IV.	—	—
Warthog ...	13.2.13	" IV.	—	—
" ...	16.2.13	" IV.	—	—

The blood of this goat was subinoculated into Goat 9, which failed to become infected. The trypanosome of which Plate 4, Group 8, No. 14, is a type, is plainly *T. uniforme*, with which it closely corresponds in the fresh blood slide.

Goat G has already ceased to show trypanosomes during routine daily examination for some three weeks at the time of writing. It is in excellent condition and has therefore probably acquired a tolerance for the trypanosome amounting possibly to immunity. It has already been noted (*Proc. Roy. Soc., B.* Vol. 85, 1912) that

this trypanosome when present alone exerts very little effect on goats. In combination, however, with *T. gambiense* the disease rapidly kills the animals with comparatively early onset of paralysis of the hind quarters.

Goat H.—Bought in fly-free and healthy district. Negative to daily blood examination for 50 days prior to the first appearance of trypanosomes in its blood, on 5th March, 1913.

Animal inoculated.	Date of inoculation.	District wherein shot.	Examination of inoculated blood.	
			2 fresh slides.	2 stained.
Giant Pig ...	28.1.13	Fly III.	—	—
" ...	28.1.13	" III.	—	—
Waterbuck ...	11.2.13	" IV.	—	—
" ...	11.2.13	" IV.	—	—
Reedbuck ...	13.2.13	" IV.	—	—
Waterbuck ...	16.2.13	" IV.	—	—
Cob ...	18.2.13	" IV.	—	—
Colobus				
Monkey ...	28.2.13	" III.	—	—

No trypanosomes were seen in the bloods inoculated into this goat.

This goat died suddenly on April 1st, 1913.

Subinoculations.

Animal.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
Rat 13 a ...	Goat H	—	—	Did not become infected.
" 14 a ...	"	—	—	" "
" 22 ...	"	—	—	" "
Monkey 4 ...	"	—	—	" "
Dog 17 ...	"	—	—	" "

The organisms thus belong to the *nanum-vivax-uniforme* group of trypanosomes.

Examination of fresh slides from Goat H revealed the presence of *T. vivax* together with another smaller and less active trypanosome.

Morphology.

The trypanosomes derived from Goat H are figured on Plate 3, Group 6, Nos. 1-10. It will be obvious at once that Nos. 1-5 represent the *T. vivax* seen in the fresh blood of the goat, and the smaller organism of Nos. 6-10 must be *T. nanum*. None of the game of which the blood was inoculated into this goat showed any obvious sign of disease.

Goat I.—Bought in fly-free and healthy district. Remained negative to daily examination for 12 days before the first appearance of trypanosomes on 25th February, 1913.

Animal.	Date of inoculation.	District wherein shot.	Examination of inoculated blood.	
			2 fresh slides.	2 stained slides.
Buffalo ...	16.2.13	Fly IV.	—	—
Hyaena ...	18.2.13	"	Not examined.	+ cf. infra.

Subinoculation for Goat I.

Animal.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
Rat 11 ...	Goat I.	8 days	20 days	A first inoculation failed to take. Showed <i>T. pecorum</i> .
" 12 ...	"	—	—	Inoculated simultaneously with Rat 11. Failed to take.
" 20 ...	"	—	—	Did not become infected.
" 21 ...	"	10 days	21 days	Showed <i>T. pecorum</i> .
" 23 ...	"	9 "	—	" "
Monkey 5 ...	"	—	—	Did not become infected.
" 6 ...	"	—	—	Ran away on 5th day. Never showed trypanosomes.
Dog 12 ...	"	9 days	15 days	Showed <i>T. pecorum</i> .
" 18 ...	"	8 "	—	" "

Morphology of trypanosomes.

Three species are recognisable.

1. A small trypanosome with a very minute or no free flagellum appearing in the subinoculated dogs and rats. (Cf. Group 7, Nos. 1-8.)

2. A trypanosome corresponding in structure with *T. uniforme* seen in Goat I. (Cf. Group 7, Nos. 9-11.)

3. A slender *T. gambiense*-like organism shown in Group 7, Nos. 12-15. Seen in Goat I.; No. 15 may well be the stumpy form of this organism.

It will be seen that No. 14 shows a posterior arrangement of the trophonucleus. The flagellar end of this specimen is unfortunately damaged.

Nothing can be decided as to identity of this trypanosome as it has not been recognised in any of the subinoculated animals. This failure to infect may be due, of course, to this trypanosome

being absent from the blood at the time of inoculation, or to its being crowded out, so to speak, by the *T. pecorum*.

Goat I is showing signs of illness, general weakness being noticeable. No paralytic symptoms have appeared so far.

Nos. 16-19 represent the trypanosomes seen in the blood of the hyaena inoculated into Goat I on 18th February, 1913. It will be seen to be a small organism resembling the *T. pecorum* which appeared in the goat as noted elsewhere, the minute free flagellum seen in Nos. 17-19 is quite compatible with *T. pecorum*.

The following animals were shot in the tsetse-free parts of Budu, Bukanga and Koki, and were inoculated into goats in the manner described above:—

- 6 Waterbuck.
- 7 Buffalo.
- 3 Reedbuck.
- 6 Impala.
- 1 Eland.
- 1 Duiker.
- 1 Lion.
- 6 Hartebeeste (topi).
- 6 Oribi.
- 2 Warthog.

All the goats remained negative.

Trypanosomes were only discovered in one antelope in the course of examination of these blood slides, *i.e.*, in the duiker shot near Kikoma (cf. Map) near Masaka. It will be seen that the animal was obtained at a considerable distance from the Lake shore and tsetse are unknown inland in this region. It is possible, however, that it may have at some time or other reached the fly zone on Lake Victoria. This organism is figured in Plate 2, Group 2, No. 1.

A comparison of the results obtained in the fly-infested and fly-free parts of the western portion of the Uganda Protectorate shows a greater predominance of trypanosomes in the former region.

In the fly area in all 52 animals were inoculated and examined microscopically, and trypanosomes were found on at least 10 occasions, *i.e.*, 19 per cent. of the animals were infected, taking the lowest possible estimate of one infected wild animal to each infected goat.

In the fly-free area, 39 animals were similarly examined, and trypanosomes found in only one instance, *i.e.*, 2.5 per cent. And this 2.5 per cent. is a far more exact estimate than is the other figure.

There is, accordingly, no reason to doubt the significance of the *Glossinae* as trypanosome carriers among game, as Tabanids, *Haemotopota*, *Muscidae*, *Chrysops*, etc., are common to both regions.

The following experiments were carried out with wild *Glossinae* caught during the tour. Wherever possible, tsetse were caught

and fed upon clean monkeys which underwent daily blood examination. A certain number of flies were subsequently dissected with a view to examining roughly the gut and proboscis.

District I.

368 *G. palpalis* caught elsewhere than at Lake Kiraro were fed upon a clean monkey without causing any infection. Of these, 101 were dissected without any flagellates being discovered.

507 Kiraro flies were fed upon a clean monkey which became infected on the 8th day after the last batch of 140 flies. 90 of these flies were dissected and proved negative to flagellates.

The reactions of the trypanosomes in the monkey will be discussed later on.

District II.

No *G. palpalis* were obtained.

117 *G. pallidipes* were fed upon clean monkeys, with negative results.

123 *G. pallidipes* were dissected, and one fly was found to contain flagellates in the gut. Proboscis negative. Salivary glands not examined. This fly had fed on the monkey. True trypanosomes were present.

District III.

210 *G. palpalis* were fed on monkeys, with negative result.

191 were dissected and no flagellates were found.

155 *G. fusca* were fed on monkeys, with negative results.

157 were dissected and flagellates were found in the gut of one fly. The proboscis was negative; the salivary glands were not examined.

The fly had not drawn blood from the monkey. True trypanosomes were seen.

District IV.

298 *G. palpalis* were fed on monkeys, with negative results.

185 were dissected, of which two contained flagellates in the gut. The probosces were negative; the salivary glands were not seen.

The flagellates of fly 1 showed crithridiae and trypanosomes and intermediate forms suggestive of a single cycle.

The flagellates of fly 2 showed scarce slender trypanosomes. Both the flies had fed upon the monkeys.

48 *G. pallidipes* were fed on monkeys, with negative results.

59 were dissected, of which one contained flagellates in the gut. Proboscis negative. Salivary glands not seen. The flagellates were of the true trypanosome structure. This fly had fed on the monkey.

15 *G. fusca* were fed upon monkeys, with negative results.

28 were dissected, of which one contained flagellates in the gut only.

The flagellates were of curious plump outline; the protoplasm stained very darkly. They were trypanosomes in type.

This fly contained no monkey blood, and therefore, very probably, had not fed.

Several of the above flagellated flies contained monkey blood on dissection. The monkeys, however, remained healthy. This may be explained either by the flagellates being in an incomplete state of development in the fly, or by the trypanosome belonging to a species which is not pathogenic to monkeys.

Diagnosis of the Trypanosome obtained from wild Tsetse caught at Kiraro Lake.

A large organism of the gambiense type, varying in the 500 individuals measured, from 17μ to 32μ in length. (Cf., Plate 4, Group 9, Nos. 1-10.) The free portion of the flagellum is well marked in the larger types. In the shorter forms this free portion is often hardly discernable. The undulating membrane is well marked. The kinetic nucleus is usually situated at a short distance from the posterior end, in the middle line.

A blue line in preparations stained with Giemsa is at times to be seen down the centre of the body. (Cf. Group 9, No. 6.) This structure has been described by Robertson and Swellengrebel in *T. brucei*, and was also seen in trypanosomes obtained recently from British East Africa. (S.S. Report, Roy., Soc. XIII.)

In the course of examination of slides from the various subinoculated animals, two forms showing the posterior arrangement of the trophonucleus were observed, one of which is figured in Group 9 to 10.

The following represents the measurements of 500 individuals of this trypanosome taken from subinoculated animals.

Animal.	17μ	18μ	19μ	20μ	21μ	22μ	23μ	24μ	25μ	26μ	27μ	28μ	29μ	30μ	31μ	32μ	Average in microns.
Monkey 1	1	3	4	8	12	33	10	11	9	2	3	2	—	—	1	1	22.56
Dog 4A	—	2	3	16	28	27	13	7	3	—	1	—	—	—	—	—	21.64
" 4B	—	—	5	11	16	22	20	11	11	1	1	1	—	—	1	—	22.46
" 10	2	4	8	16	14	18	16	10	7	3	1	1	—	—	—	—	21.84
Rat 8 ...	—	—	1	3	5	8	24	18	11	11	9	7	3	—	—	—	24.31
Per cent.	.6	1.8	4.2	10.8	15	21.6	16.6	11.4	8.2	3.4	3	2.2	.6	—	.4	.2	22.5

These random measurements are not of much value in making a differential diagnosis. The minimal measurement is somewhat larger than those generally given for *T. gambiense*, *brucei* and *rhodesiense*, while the average measurements correspond. It may be noted that the *T. gambiense* received from wild situtunga antelope on the Islands of Lake Victoria showed a minimum measurement of 17μ in 1,000 individuals measured.

The following table deals with the subinoculated experiments and animal reactions.

Animal.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
Monkey 1...	Wild <i>G. palpalis</i> at Lake Kiraro.	?	52 days from first appearance of T's.	
" 2...	Monkey 1	?	31 days	Shown haemoglobin-urea.
Dog 4 ...	"	7 days	32 "	
" 10 ...	Dog 4	6 "	22 "	Shown haemoglobin-urea and corneal opacity.
Guinea-pig 1	Dog 10	14 "	38 "	
Rat 7 ...	"	6 "	15 "	
" 8 ...	"	6 "	17 "	
Bullock 6 ...	Monkey 2	?	24 "	

The virulence of rats is greater than that shown by *T. gambiense* and the disease in the bullock was extraordinarily rapid. In the case of this last, however, there is room to doubt whether the trypanosomes were the sole cause of death.

The general scale of virulence in the above experiments corresponds in an organism of the *brucei* and *rhodesiense* type. The haemoglobinuria was much more marked in the dog than in the monkey. The animal had several relapses of this symptom, with intervening periods of clear urine. No pyroplasma were seen in the blood. This interesting symptom may have been due to the lighting up of a latent pyroplasmosis, but such an occurrence has never been seen in any of the numerous dogs at Mpumu which died of trypanosomiasis. The clouding of the corneae of this dog was also very striking.

It was impossible to investigate the behaviour of the trypanosome in laboratory-bred *G. palpalis*, owing to the temporary moveal of the laboratory to Kampala and the impossibility of importing tsetse. The origin of the strain, however, excludes *T. evansi* from the diagnosis.

The occurrence of posterior nuclear forms, in three of the trypanosomiasis described in this paper, is of considerable interest. Since the separation of *T. rhodesiense* as a species, largely upon the strength of the occurrence of such posterior-nuclear forms, several observers have found this peculiarity in different strains of typanosomes. It is possible, of course, that Stephens and Fantham are correct in claiming that this morphological peculiarity is diagnostic of their trypanosome, and that the posterior-nuclear organisms described by other observers under other names are in reality *T. rhodesiense*. It is equally possible, however, that the same peculiarity is shared by other so-called species. With increasing knowledge on the subject of game trypanosomes throughout the world, it is probable that the phenomenon will prove more general than is assumed at

present. As emphasised in a previous paper, it is very difficult to distinguish between mere varieties of strain and true species. The whole trypanosome group would appear to be in an extremely unsettled state in which strongly adverse or strongly favourable conditions may effect drastic changes in a given strain of organisms. In dealing with the differential diagnosis of these parasites, it is imperative to recollect that the natural means of propagation in the vast majority of trypanosomes is by biting insects. In the insect the trypanosome undergoes a most important phase of its race history, and without this sojourn in the insect, the species could not be propagated in nature. It is illogical, therefore, to devote undivided attention to the blood phases of a trypanosome strain which is propagated by the syringe from host to host, often for a period of years. In this way all kinds of variations are liable to occur and to be preserved, owing to the removal of the natural control exercised by the probable regulatory processes undergone in the biting insect.

With regard to the coincidence of distribution between certain species of tsetse and of game, my experience, though limited, points to the following conclusions. As mentioned above, *G. fusca* is chiefly confined to forest areas, being found throughout the great forests bordering the Durro River and its branches, and also in the nullas along the north-eastern spurs of Mt. Ruwenzori and the Bwamba country. The game of these regions comprises elephant, giant and bush pig, buffalo, hippo, bushbuck, and various Anthropoideae. In my experience, elephant, giant pig, and bushbuck are the most constant frequenters of these *fusca* haunts, signs of their presence being constantly met with in the larger forests. Buffalo seem to be less regular visitors, except in certain regions where strips of forest alternate with and often surround the open grassy spaces where these animals graze.

The parts of Toro around Katwe Mahokya and Kasesse consist of short grass plains running up to the elephant-grass country around the Hima river. The whole of this area swarms with buffalo. *G. palpalis*, *pallidipes* and *morsitans* are found, but *fusca* appears to be the first met with in the forests to the north and east of the Hima river. Hippo, where they occur, doubtless constitute the important food supply of *fusca*, but their range is limited to certain lakes and streams. It would seem that the primary requirement of *G. fusca* is forest, and that they feed indiscriminately on the game frequenting these forests, of which elephant, giant pig, bushbuck and buffalo are the most regular victims. This tsetse, as has often been reported, is especially active towards dusk, and seems to prefer animal to human blood.

G. palpalis in the parts visited also occurs in the forested area only, differing from *fusca* in its confinement to the neighbourhood of water. In considering its food supply man will take a prominent place, and hippo are very often found in the haunts of this tsetse.

G. pallidipes occurs throughout the buffalo district referred to above, and is found chiefly in open forest areas and in scrub country. In the neighbourhood of suitable streams it will often be found in conjunction with *G. palpalis*. It is also met with in isolated thickets, a considerable distance from the scrub which borders the open tracts. It thus comes into contact with antelope such as cob and reedbuck, which abound on the plains, and especially with waterbuck, which frequent the scrub around the edges of the plains, feeding out thence into the open among the herds of smaller game. Buffalo will also form an important part of the food supply of this tsetse, as they occur in large numbers throughout the *pallidipes* area.

Concerning *G. morsitans* I can say nothing, as I did not meet with this fly during my investigations. Dr. van Someren, however, travelling in the same district in 1910, reported its occurrence in close connection with buffalo.

The distribution of *G. pallidipes*, *palpalis* and *fusca* in the Semiliki valley bears out the above conclusions, and points to *G. pallidipes* as the species with the least restricted range, and consequently, in open scrub country, the most important species in the spread of trypanosomiasis among game.

There is little of interest to record concerning other biting flies. A species of *hippobosca*, first taken by van Someren in this region, was seen on almost every antelope shot in this part. It was taken in greatest numbers on waterbuck, and once as many as 12 were caught around one animal. They were taken also on a reedbuck shot out on the plains, far away from the scrub. This fly was never seen on game shot in the Semiliki valley, perhaps owing to the much lower altitude of this district, the difference in elevation being some 3,000 to 4,000 feet. Dr. van Someren referred to a successful transmission of a cattle trypanosome by means of this fly, of which data are not available. It is thus possible that this species may play a part in the spread of trypanosomes, and in tsetse-free districts may account for the existence of disease amongst cattle. I did not see it on cattle, however, and its absence from the buffalo is noteworthy, indicating a preference for longer-haired game. Dissection of 15 individuals of this fly proved negative to flagellates.

CONCLUSIONS.

1. A considerable portion of the wild game in the fly districts of the Western Province of the Uganda Protectorate is infected with trypanosomiasis.

2. Certain of these trypanosomes have suspicious resemblance to the human trypanosomes, *T. gambiense* and *T. rhodesiense*. Others are known to be exceedingly pathogenic to domestic animals.

3. The tsetse of these parts also contain flagellates which it is highly probable are derived from wild animals.

4. The population of the fly districts is scanty, and the greater part has recently been moved to fly-free parts.

5. It is reasonable to hope that, as regards the spread of human trypanosomiasis, the removal of the people from the infected districts will suffice, and this measure will simultaneously prevent native cattle being exposed to the fly bite. The majority of the fly area is practically uninhabited country.

6. The alternative of destroying the game, and so of abolishing what is doubtless a permanent trypanosome reservoir, would be a gigantic and almost impossible undertaking in this region. In considering such a proposal elephant must, of course, be included, as must, also, hippo, situtunga, bush pig and hyæna, all of which are difficult to eradicate. The scarcity of people is a serious objection to such a course, as is the difficult nature of the country. It would be well to await the trial of this expedient under more favourable conditions before undertaking so drastic a measure under severe natural handicaps.

7. It would appear inadvisable to take any measures to protect the existing game and thereby encourage an increase in their numbers. On the other hand, it is inadvisable to permit natives to hunt in the fly districts. The ideal arrangement would be to make the fly districts prohibited areas, and in the region under consideration this is feasible to a far greater extent than would be the case in other parts of the Protectorate.

Special attention must be directed to the Mahokya district in order to stamp out *G. pallidipes* from the immediate neighbourhood of the main road.

EXPLANATION OF PLATES.

Group 1.

Trypanosomes derived from Goat A.

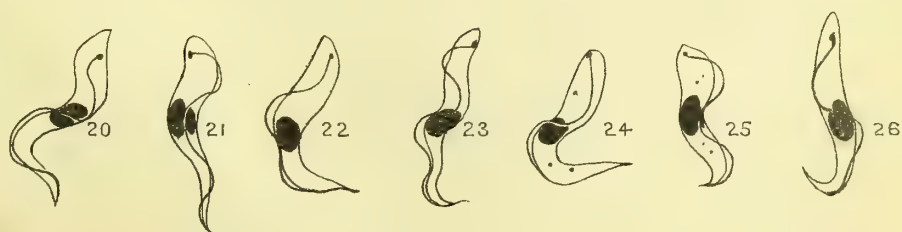
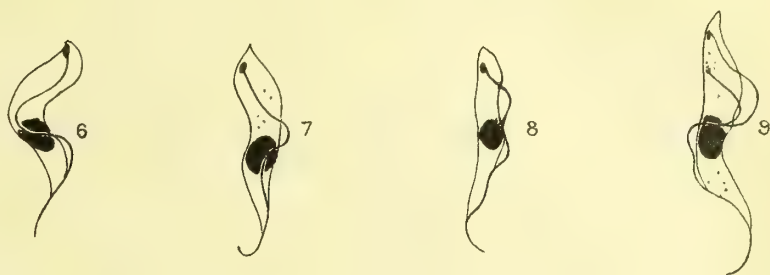
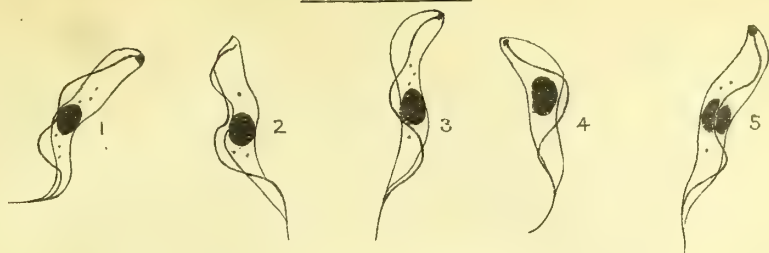
Drawn at a magnification of 2,000 diams.

- | | | |
|--|-----|---------------------|
| Nos. 1-8.—From blood of Goat A | ... | <i>T. uniforme.</i> |
| No. 9.—Dividing form, from Goat A | ... | <i>T. uniforme.</i> |
| Nos. 10-14.—From blood of Goat A | ... | <i>T. pecorum.</i> |
| Nos. 15-23.—From blood of Dog 11 | ... | <i>T. pecorum.</i> |
| Nos. 24-26.—From blood of Goat 4 | ... | <i>T. pecorum.</i> |
| Nos. 27-32.—From blood of Bullock 4 | ... | <i>T. pecorum.</i> |
| No. 33.—From blood of Goat A | ... | c.f. text. |
| Nos. 34-35.—From blood of Dog 6 | ... | c.f. text. |
| Nos. 36-49.—From blood of Buffalo of 4/1/13. | | |

Group 2.

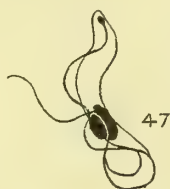
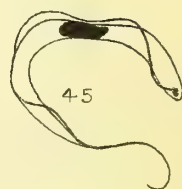
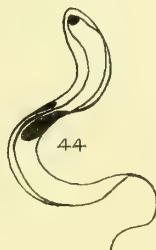
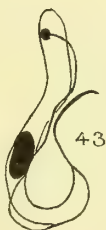
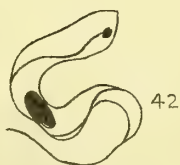
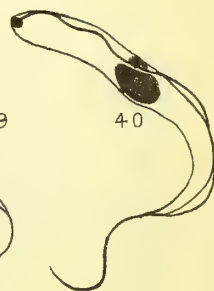
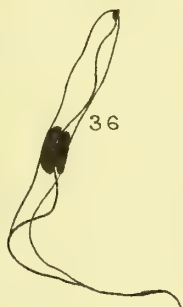
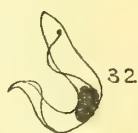
Trypanosome from Duiker. Drawn at approx. 1,800 diam. magnification.

PLATE I.



GROUP I.

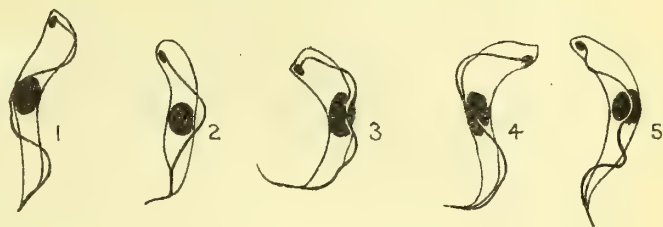
PLATE I (cont.^d)



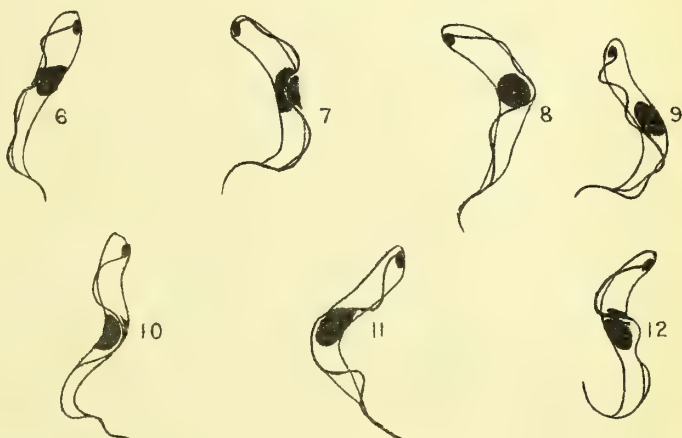
GROUP I (cont.^d)



PLATE II.



GROUP 2.

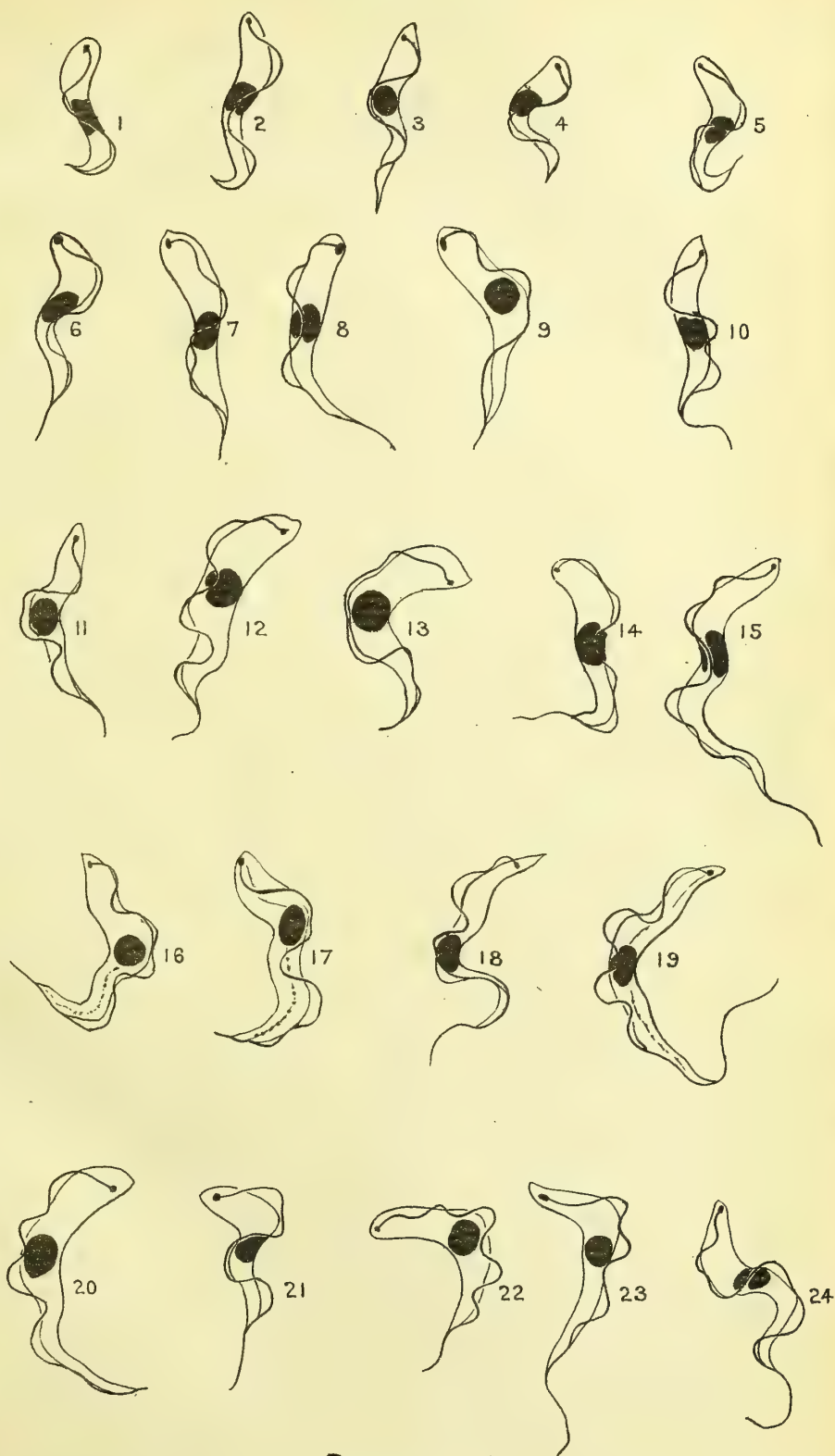


GROUP 3.



GROUP 4.

PLATE III.



GROUP 5.

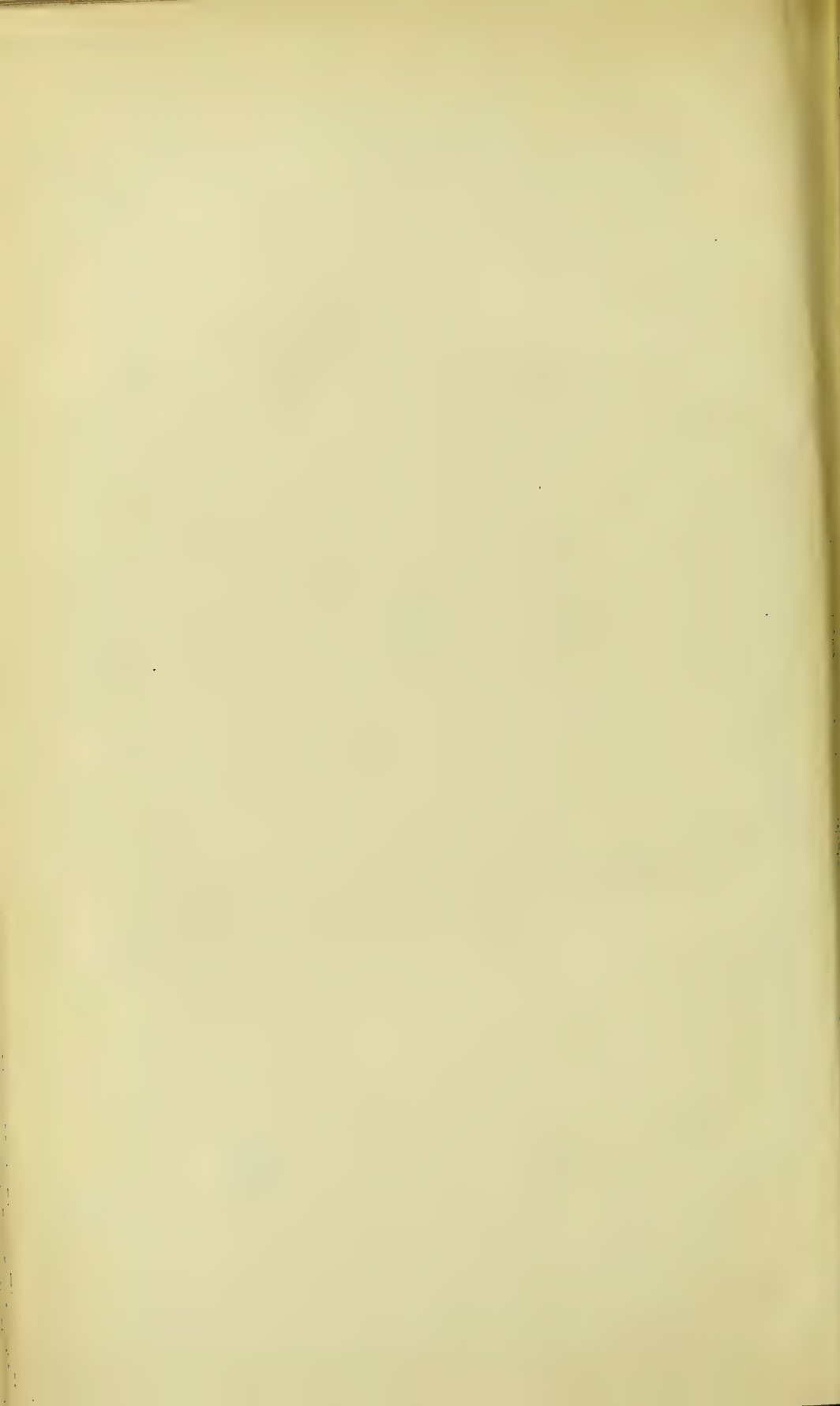
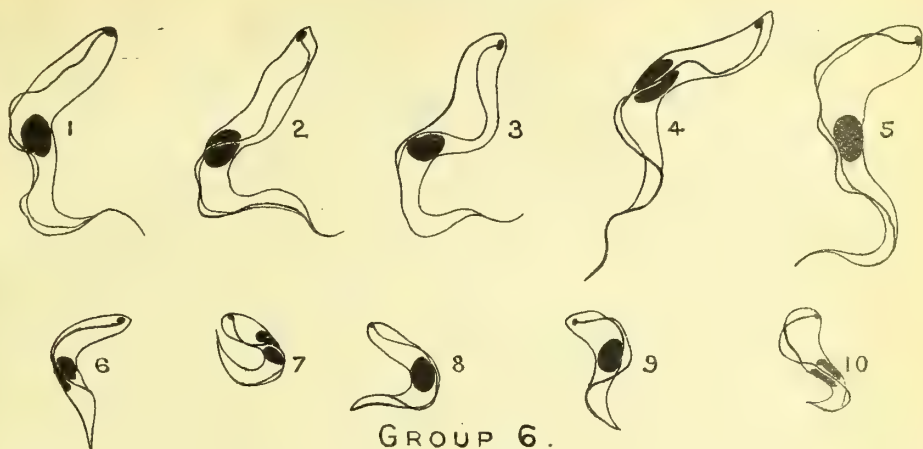


PLATE III (cont^d)



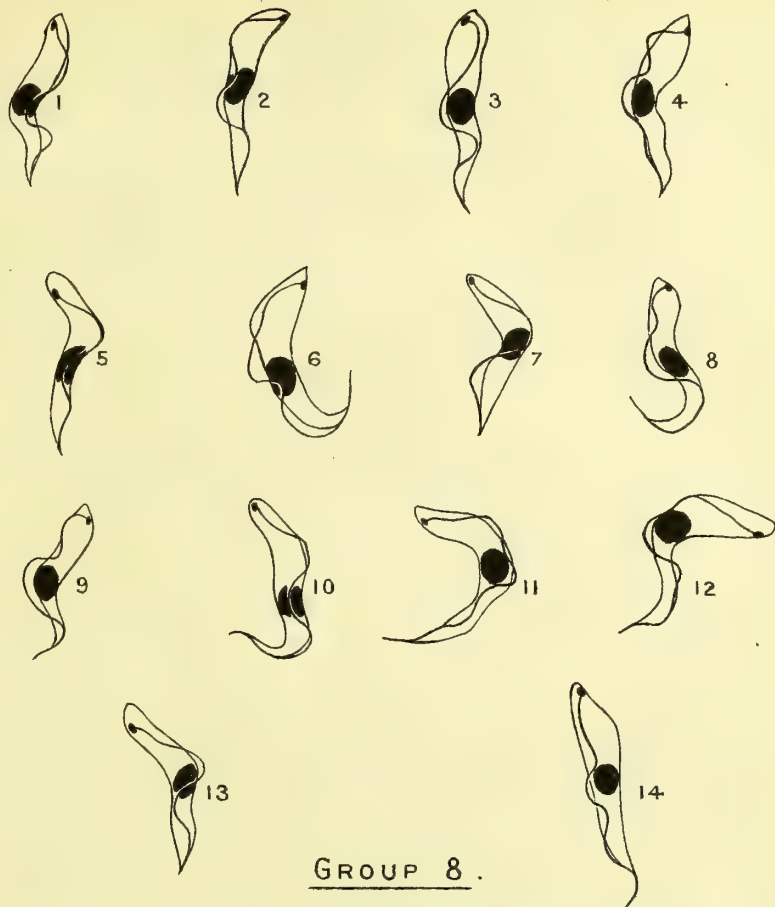
GROUP 6.

PLATE IV.

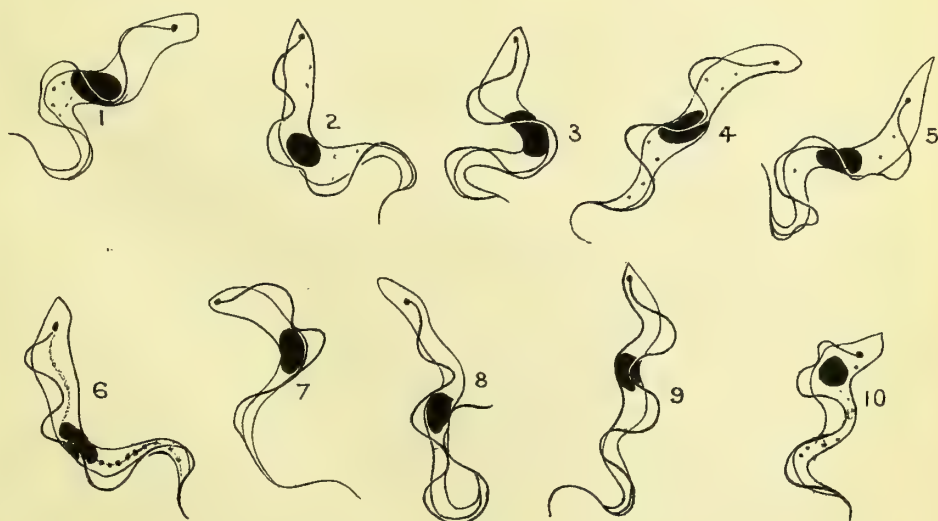


GROUP 7.

PLATE IV (cont'd)



GROUP 8.



GROUP 9.



Group 3.

Trypanosomes from waterbuck of 17/12/12. (x 2,000.)

Nos. 1-12.—*T. uniforme*-like organism.

No. 13.—*T. vivax*-like organism.

Group 4.

Trypanosomes from Goat D. (x 2,000.)

Nos. 1-2.—From blood of Goat D.

No. 3.—From blood of Goat 2.

Nos. 4 and 8.—From blood of Goat 2.

Nos. 5, 6, 7, 9.—From blood of Goat D.

Group 5.

Trypanosomes derived from Goat E. (x 2,000.)

Nos. 1-11.—From blood of Goat E.

Nos. 12-15.—From blood of Dog 5.

Nos. 16-19.—From blood of Guinea-pig 3.

Nos. 20-24.—From blood of Monkey 3.

Group 6.

Trypanosomes from Goat H. (x 2,000.)

Nos. 1-10.—From blood of Goat H.

Group 7.

Trypanosomes from hyæna and from Goat I. (x 2,000.)

Nos. 1-4.—From blood of Goat I.

Nos. 5-8.—From blood of Dog 12.

Nos. 9-11.—From blood of Goat I.

Nos. 12-15.—From blood of Goat I.

Nos 16-19.—From blood of hyæna of 18/2/13.

Group 8.

Trypanosomes derived from blood of Goats F and G. (x 2,000.)

Nos. 1-13.—From blood of Goat F.

No. 14.—From blood of Goat G.

Group 9.

Trypanosomes derived from Monkey infected by Kiraro flies.
(x 2,000.)

Nos. 1-10.—From blood of Monkey.

ANTELOPE INFECTED WITH *TRYPANOSOMA* *GAMBIENSE*.*

By Captain A. D. FRASER, R.A.M.C., and Dr. H. L. DUKE.

(Communicated by Sir John Bradford, Sec. R.S. Received
December 2, 1911—Read January 25, 1912.)

The Sleeping Sickness Commission of the Royal Society, Uganda, 1908-10, showed that waterbuck, bushbuck, and reedbuck could be readily infected with a human strain of *Trypanosoma gambiense*, and that clean laboratory-bred *Glossina palpalis* were capable of transmitting the virus from the infected antelope to susceptible animals.

In the present paper, observations which were made upon these antelope during the eight months subsequent to the Commission's departure from Uganda are recorded. Experiments are also described which show that the duiker—another species of antelope common in most parts of Uganda—can also be similarly infected with a human strain of *T. gambiense*. As regards the antelope employed by the Commission, six of the nine remained in apparently excellent health in April, 1911—roughly, a year after they were infected.

Until Bushbuck 2428 escaped from the kraal, and Bushbuck 2372 died 338 days after its infection as the result of an accident, they had also been healthy. A *post-mortem* examination was made immediately after death in the case of Bushbuck 2372, but no evidence of trypanosomiasis was found.

Reedbuck 2427 appeared to be perfectly healthy for 200 days after it had been infected. It then died suddenly. At the *post-mortem* examination performed immediately after death the prescapular glands were found to be the size of a hazel-nut. On section they were hæmorrhagic. There were numerous petechiæ on the mucous membrane of the mesentery. The mucous membrane of the fourth stomach also showed many petechiæ. Microscopical examination of smears made from the various organs was negative. It is, therefore, impossible to say what the cause of death was in the case of this buck.

With the view of ascertaining how long the antelope remained infected, investigations were carried out on the following lines:—

1. Feeding laboratory-bred *Glossina palpalis* for several days on the antelope and subsequently endeavouring to infect a healthy susceptible animal.
2. Dissecting these flies and examining them for flagellates.
3. Injecting blood of the buck into animals susceptible to *T. gambiense* infection.

Table I. gives the detailed results obtained by the first of these methods. The number of days which elapsed from the date

* Reprinted from the Proceedings of the Royal Society, Series B, vol. 84 1912, pp. 484-492.

on which the buck was infected until the commencement of the experiment is given.

"Hereditary transmission" flies indicates that the flies before being put upon the antelope had been fed for 30 days upon healthy monkeys to ascertain if laboratory-bred flies which had never fed upon an infected animal could give rise to an infection. As it has been suggested that flies were most readily infected when their first feed was upon an infected animal, these flies were used with the view of obtaining evidence on this point, control experiments being at the same time made with laboratory-bred flies which had not fed before they were put upon the antelope. Although it will be noted that no infection occurred among the "hereditary transmission" flies, where as the control flies some times became infected, the numbers of the flies used are too small to allow of any conclusions being arrived at.

It will be noted that flies which were fed on Bushbuck 2372, 315 days after it had been infected with a human strain of *T. gambiense*, became infected and successfully transmitted the disease to a healthy monkey.

Table II. gives the results of the dissections of laboratory-bred *Glossina palpalis* which had fed on the infected antelope.

The experiments recorded in Tables I. and II. are summarised and grouped according to the length of time the antelope had been infected in Table III.

Table I.—Giving the Results of feeding Laboratory-bred *Glossina palpalis* on Antelope infected with *T. gambiense*.

Ex- peri- ment No.	No. of flies.		No. of days after original infection of antelope.	No. of days flies fed on antelope.	No. of days before flies became in- fective.	Result.		Length of experi- ment, in days.	Remarks.
	On 1st day.	On 30th day.				Posi- tive.	Nega- tive.		
Bushbuck, Expt. 2328.									
7	?	?	134	5	25	+		32	Experiment carried out by by Dr. van Someren.
215	115	79	202	10			—	79	
402	53	18	264	11			—	64	"Hereditary transmission" flies.
413	49	29	264	11			—	64	Control to Expt. 402.
538	21	16	306	7			—	48	"Hereditary transmission" flies.
539	39	31	306	7			—	62	Control to Expt. 538.
647	52	46	342	6			—	41	
Bushbuck, Expt. 2371.									
89	?	?	126	3	50	+		59	Experiment carried out by Dr. van Someren.
216	90	64	173	10			—	78	
404	50	21	235	11			—	63	"Hereditary transmission" flies.
405	43	31	235	11	38	+		51	Control to Expt. 404.
543	33	17	279	9			—	60	"Hereditary transmission" flies.
544	71	44	279	9			—	60	Control to Expt. 543.
643	73	49	311	5			—	42	

Table I.—*continued.*

Ex- peri- ment No.	No. of flies.		No. of days after original infection of antelope.	No. of days flies fed on antelope.	No. of days before flies became in- fective.	Result.		Length of experi- ment, in days.	Remarks.
	On 1st day.	On 30th day.				Posi- tive.	Nega- tive.		
Bushbuck, Expt. 2372.									
88	?	?	123	3	39	+		48	Experiment carried out by Dr. van Someren.
218	72	59	171	9	34	+		47	
480	79	47	253	7			-	64	
607	85	61	208	8	25	+		33	
658	64	48	315	9	38	+		38	
Bushbuck, Expt. 2428.									
106	?	?	116	2			-	67	Experiment carried out by Dr. van Someren.
222	20	20	164	9	40	+		49	
Reedbuck, Expt. 2357.									
51	92	18	131	7			-	77	" Hereditary transmission " flies. Control to Expt. 528.
189	101	62	173	11	44	+		59	
469	83	34	263	9	31	+		43	
528	41	20	288	8			-	57	
529	70	55	288	8	34	+		43	
631	97	56	322	6			-	41	
669	119	63	336	12			-	35	
Reedbuck, Expt. 2359.									
52	170	64	113	7	38	+		55	Control to Expt. 400. " Hereditary transmission " flies.
190	89	76	155	11	32	+		44	
398	50	34	231	12	36	+		49	
400	49	13	231	12			-	63	
530	54	19	270	8			-	61	
642	33	29	306	5			-	40	
Reedbuck, Expt. 2427.									
97	60	53	117	8	34	+		46	
254	72	52	177	11	29	+		49	
Reedbuck, Expt. 2431.									
98	81	41	119	7	32	+		43	Control to Expt. 401. " Hereditary transmission " flies.
268	60	51	184	10	36	+		50	
399	43	29	224	12			-	63	
401	25	4	224	12			-	63	
598	88	44	288	9			-	50	
656	66	30	306	11			-	40	
Waterbuck, Expt. 2378.									
6	?	?	105	5	25	+		32	Experiment carried out by Dr. van Someren.
217	168	53	173	10			-	68	
406	54	0	237	9			-	19	
471	96	9	251	7			-	62	
488	45	28	259	8			-	59	
523	36	24	270	7			-	62	
622	62	15	306	7			-	42	

Table II.—Giving the Results of the Dissection of Laboratory-bred *Glossina palpalis* which had Fed on Antelope infected with *T. gambiense*.

Experiment No.	No. of flies used in experiment.	No. of flies dissected.	No. of infected flies found.	Percentage of infected flies.	Result of experiment.	Remarks.
Bushbuck, Expt. 2,328.						
7	?	0	0		+	Flies not dissected.
215	115	79	0		—	
402	53	42	0		—	
403	49	42	0		—	
538	21	21	0		—	
539	39	33	0		—	
647	52	51	0		—	
Bushbuck, Expt. 2,371.						
89	?	27	0		+	
216	90	61	0		—	
404	50	33	0		—	
405	43	41	0		+	
543	33	26	0		—	
544	71	66	0		—	
643	73	51	0		—	
Bushbuck, Expt. 2,372.						
88	?	29	1	3.45	+	
218	72	37	1	2.7	+	
480	79	44	0		—	
607	84	70	1	1.4	+	
658	64	59	2	3.4	+	
356	36	36	0		—	Experiment lasted 12 days. " 14 "
357	38	38	0		—	
Bushbuck, Expt. 2,428.						
106	?	14	0		—	
222	20	20	2	10	+	
Reedbuck, Expt. 2,357.						
51	92	30	0		—	
189	101	46	4	8.6	+	
469	83	53	5	9.4	+	
528	41	31	0		—	
529	70	64	3	4.7	+	
631	97	77	0		—	
669	119	65	0		—	
Reedbuck, Expt. 2,359.						
52	170	122	0		+	
190	89	61	5	8.1	+	
398	50	49	3	6.1	+	
400	49	40	0		—	
530	54	23	0		—	
642	33	30	0		—	
Reedbuck, Expt. 2,427.						
97	60	27	1	3.7	+	
254	72	49	1	2.0	+	
322	28	28	0		—	Experiment lasted 9 days. " " 13 "
323	30	30	1	3.3	—	

Table II.—*continued.*

Experi- ment No.	No. of flies used in experi- ment.	No. of flies dis- sected.	No. of in- fected flies found.	Per- centage of infected flies.	Result of experi- ment.	Remarks.
Reedbuck, Expt. 2,431.						
98	81	8	0	3·7	+	
268	60	53	2		+	
399	43	36	0		—	
401	25	14	0		—	
598	88	45	0		—	
656	66	35	0		—	
Waterbuck, Expt. 2,378						
6	?	0	0	3·8	+	
217	168	53	2		—	
406	54	36	0		—	
471	96	18	0		—	
488	45	44	0		—	
523	36	31	0		—	
622	62	20	0		—	

Table III.—Summarising Experiments of Tables I. and II. and grouping them according to Length of Time the Antelope had been infected.

Species of antelope.	Days after original infection of antelope.	No. of experi- ments.	No. of posi- tive experi- ments.	No. of flies.		No. of flies dis- sected.	No. of infected flies found.
				On 1st day.	On 30th day.		
Bushbuck 2328	100-200	1	1	?	?	0	0
" 2371	"	2	1	?	?	88	0
" 2372	"	2	2	?	?	66	2
" 2428	"	2	1	?	?	34	2
Reedbuck 2357	"	2	1	193	80	76	4
" 2359	"	2	2	259	140	183	5
" 2427	"	4	2	190	105	134	3
" 2431	"	4	2	209	125	111	2
Waterbuck 2378	"	2	1	?	?	53	2
Bushbuck 2328	200-300	3	0	217	126	163	0
" 2371	"	4	1	197	113	166	0
" 2372	"	4	1	238	108	188	1
Reedbuck 2357	"	3	2	194	118	148	8
" 2359	"	3	1	151	66	112	3
" 2431	"	2	0	154	74	80	0
Waterbuck 2378	"	4	0	231	61	129	0
Bushbuck 2328	300-342	3	0	112	93	105	0
" 2371	"	1	0	73	49	51	0
" 2372	"	1	1	64	48	59	2
Reedbuck 2357	"	2	0	216	119	142	0
" 2359	"	1	0	33	29	30	0
Waterbuck 2378	"	1	0	62	15	20	0

It will be seen that positive experiments were obtained from all the buck (nine examined) when the flies were fed upon them before 200 days had elapsed from the date of the antelope's infection. When more than 200 days had elapsed four of the seven buck examined yielded positive results.

The results of all experiments are shown in Table IV.

Table IV.—Giving Results of Experiments from all Antelopes.

Interval in days after infection of antelope.	No. of experiments.	No. of positive experiments.	No. of flies dissected.	No. of flies found infected.	Percentage of infected flies.
100-200... ..	21	13	745	20	2·7
200-300... ..	23	5	986	12	1·2
300-342... ..	9	1	407	2	0·5
Totals	53	19	2,138	34	1·6

It appears from the above table that as the interval after the infection of the antelope increases, the percentage of positive transmission experiments and of flies which become infected with flagellates after having fed on the buck diminishes. This diminution becomes still more striking when these results are compared with those recorded by the Commission of experiments carried out soon after the antelope were infected. (Of the 24 experiments carried out by the Commission 17 were positive, 1,722 flies were dissected, and 6·9 per cent. were found to be infected.)

The results of injecting blood of these antelope into susceptible animals are shown in Table V. (p. 491).

It is seen that the injection of a small quantity of the blood of Bushbuck 2372, 327 days after it had been infected with *T. gambiense*, produced an infection in a white rat. This, however, was the only positive result which was obtained. Three injections were carried out from Waterbuck Experiment 2378—on one occasion 5 c.c. of blood was injected—and all were negative. It will be remembered that the Commission found it easy to produce infections in susceptible animals by injecting the blood taken from these antelope soon after they were infected.

Table V.—Giving Results of injecting the Blood of Antelope infected with *T. gambiense* into susceptible Animals.

Antelope.	No. of days after infection of antelope.	Animal used.	Quantity of blood injected in c.c.	Result.	Remarks.
Reedbuck 2427	200	Monkey	2	Inconclusive.	Monkey died. Negative for 9 days.
Waterbuck 2378	306	White rat	1	—	
" "	316	Monkey	5	—	
" "	316	White rat	1	—	
Bushbuck 2328	355	"	1½	—	
" 2371	330	"	1	—	
" 2372	327	"	1	+	Trypanosomes appeared in rat on the 12th day.
Reedbuck 2357	345	"	1	—	
" 2357	327	"	1	—	
" 2431	320	"	1	—	
Bushbuck 2372	338	Monkey	5	Inconclusive.	Monkey died before trypanosomes could have appeared.

Can a Duiker be Infected with a Human Strain of T. gambiense?

Experiment 99, Duiker.—On August 30, 1910, 3 c.c. of this buck's blood were injected subcutaneously into a normal monkey to ascertain if the antelope naturally harboured trypanosomes. The monkey's blood was examined regularly for a month. No trypanosomes appeared in its blood, the monkey remaining healthy.

For nine days (January 25 to February 2, 1911, inclusive) laboratory-bred *G. palpalis* known to be infected with a human strain of *T. gambiense* were fed upon the buck.

On February 4, the tenth day after the infected flies first fed upon the antelope, *T. gambiense* appeared in fair numbers in its blood.

On February 10 and 11, 1911, 119 clean laboratory-bred *G. palpalis* were fed upon the duiker. These flies were subsequently fed on a normal monkey, which they infected after 28 days had elapsed from the date of their first feed on the buck. Of 42 flies which were dissected, two were found to be infected with flagellates.

Remarks.—The duiker was free from trypanosomes inoculable into a monkey on its arrival at the laboratory.

T. gambiense appeared in the buck's blood on the tenth day after infected flies had fed upon it, and clean laboratory-bred flies successfully transmitted the infection to a healthy susceptible monkey.

Conclusions.

1. Antelope may remain in apparently perfect health for a year after having been infected with a human strain of *T. gambiense*.

2. One antelope was still capable of infecting clean laboratory-bred *G. palpalis* 315 days after it had been infected.

3. A small quantity of blood taken from one antelope 327 days after its infection was proved by inoculation into a white rat to be infective.

4. At the interval after the infection of the antelope increases their infectivity, as tested by "cycle" transmission experiments, dissection of flies which have fed upon them, and by the injection of the buck's blood into susceptible animals, appears to diminish.

5. A duiker was infected with a human strain of *T. gambiense* by feeding infected *G. palpalis* upon it.

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OF THE

SLEEPING SICKNESS COMMISSION

OF THE

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Prof. R. Newstead and Dr. J. B. Davey.

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I.—THE MORPHOLOGY OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND.*

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Majors DAVID HARVEY and A. E. HAMERTON, D.S.O., R.A.M.C.; Dr. J. B. DAVEY, Nyasaland Medical Staff; and Lady BRUCE, R.R.C.

(Scientific Commission of the Royal Society, Nyasaland, 1911-12.)

(Received May 24, 1912.)

[Plates 1 and 2.]

INTRODUCTION.

This species, like *Trypanosoma brucei* and *T. gambiense*, is characterised by showing two distinct forms—the long and slender, and the short and stumpy. These are not sharply divided from one another, but are connected by intermediate forms, so as to form an unbroken series, or curve, from the shortest to the longest.

This strain of the trypanosome was obtained from a native woman suffering from “Kaodzera,” the so-called Sleeping Sickness of Nyasaland.

A. *Living, Unstained.*

The movements of the Nyasaland trypanosome resemble *T. brucei* and *T. gambiense* in being non-translatory either in fresh blood or in dilutions with citrate solution. Under dark background illumination, however, with a higher degree of dilution, some translatory movement is seen, the trypanosomes swimming slowly across the field.

B. *Fixed and Stained.*

The blood films were fixed, stained, and measured as previously described in the “Proceedings.”†

Length.—The following table gives the length of this trypanosome as found in man, monkeys, goats, sheep, dogs, guinea-pigs, and white rats—1220 trypanosomes in all. From this it will be seen that it measures, on an average, rather more than *T. brucei* or *T. gambiense*, but the difference is so small as to be probably of no value as a means of separating these species.

* Reprinted from the Proceedings of the Royal Society, Series B, vol. 85, pp. 423-433.

† ‘Roy. Soc. Proc,’ 1909, B, vol. 81, pp. 16 and 17. (S.S. Reports, No. X., p. 12.)

Table I.—Measurements of the Length of the Trypanosome of the Human Trypanosome Disease of Nyasaland.

Date.	No. of Experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1.9.10	—	Man (P.R.)	Osmic acid	Giemsa	23·6	28·0	18·0
1.9.10	—	"	"	"	24·3	30·0	18·0
1.9.10	—	"	"	"	23·9	29·0	20·0
29.1.12	24	Monkey	"	"	22·1	30·0	17·0
6.2.12	24	"	"	"	18·0	21·0	16·0
8.2.12	24	"	"	"	19·9	29·0	16·0
15.2.12	24	"	"	"	20·2	23·0	17·0
19.2.12	234	"	"	"	23·0	31·0	18·0
15.2.12	142	Goat	"	"	24·3	28·0	21·0
9.2.12	163	Sheep	"	"	25·0	29·0	20·0
9.2.12	163	"	"	"	25·1	29·0	22·0
17.2.12	164	"	"	"	24·6	29·0	21·0
19.1.12	11	Dog	"	"	28·3	34·0	23·0
25.1.12	14	"	"	"	25·9	32·0	17·0
29.1.12	14	"	"	"	22·7	30·0	16·0
5.2.12	111	"	"	"	23·4	28·0	18·0
6.2.12	111	"	"	"	27·1	33·0	18·0
7.2.12	111	"	"	"	25·1	31·0	17·0
8.2.12	111	"	"	"	24·6	31·0	18·0
12.2.12	111	"	"	"	23·7	30·0	17·0
9.2.12	111	"	"	"	24·5	29·0	15·0
13.2.12	111	"	"	"	20·3	27·0	16·0
15.2.12	111	"	"	"	24·1	32·0	16·0
14.2.12	111	"	"	"	23·2	29·0	17·0
14.2.12	111	"	"	"	22·5	33·0	17·0
19.1.12	13	Guinea-pig	"	"	19·0	28·0	14·0
25.1.12	13	"	"	"	24·6	36·0	16·0
29.1.12	13	"	"	"	22·2	28·0	15·0
12.2.12	13	"	"	"	23·1	29·0	16·0
19.2.12	165	"	"	"	25·0	30·0	16·0
15.2.12	13	"	"	"	21·7	28·0	16·0
19.12.11	38	Rat	"	"	26·6	33·0	20·0
22.1.12	38	"	"	"	27·7	33·0	18·0
22.1.12	37	"	"	"	26·7	33·0	17·0
29.1.12	36	"	"	"	28·2	31·0	23·0
1.2.12	36	"	"	"	26·4	33·0	22·0
6.2.12	36	"	"	"	28·3	34·0	18·0
6.2.12	36	"	"	"	27·2	33·0	18·0
1.2.12	36	"	"	"	27·2	35·0	21·0
6.2.12	36	"	"	"	28·2	31·0	19·0
20.2.12	236	"	"	"	21·6	30·0	18·0
20.2.12	236	"	"	"	22·7	29·0	18·0
22.2.12	235	"	"	"	25·5	29·0	18·0
21.2.12	235	"	"	"	25·1	29·0	19·0
21.2.12	236	"	"	"	25·5	30·0	19·0
21.2.12	236	"	"	"	25·1	34·0	19·0
22.2.12	235	"	"	"	24·8	31·0	19·0
22.2.12	236	"	"	"	24·7	33·0	18·0
22.2.12	236	"	"	"	24·0	34·0	19·0
22.2.12	235	"	"	"	23·5	28·0	19·0
23.2.12	235	"	"	"	24·0	33·0	18·0
23.2.12	235	"	"	"	25·2	32·0	19·0
23.2.12	236	"	"	"	25·5	31·0	19·0
24.2.12	235	"	"	"	24·4	29·0	19·0
1.2.12	36	"	"	"	28·7	34·0	19·0

Table I.—*continued.*

Date.	No. of Experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
23.2.12	236	Rat ...	Osmic acid	Giemsa	24·5	34·0	18·0
24.2.12	236	" ...	"	"	20·9	29·0	18·0
24.2.12	235	" ...	"	"	25·4	32·0	20·0
24.2.12	236	" ...	"	"	22·2	30·0	18·0
26.2.12	235	" ...	"	"	21·8	31·0	16·0
26.2.12	236	" ...	"	"	21·5	30·0	18·0
					24·1	36·0	14·0

The average length of the trypanosome of the human trypanosome disease of Nyasaland in man and other species of animals, taken from Table I., is as follows:—

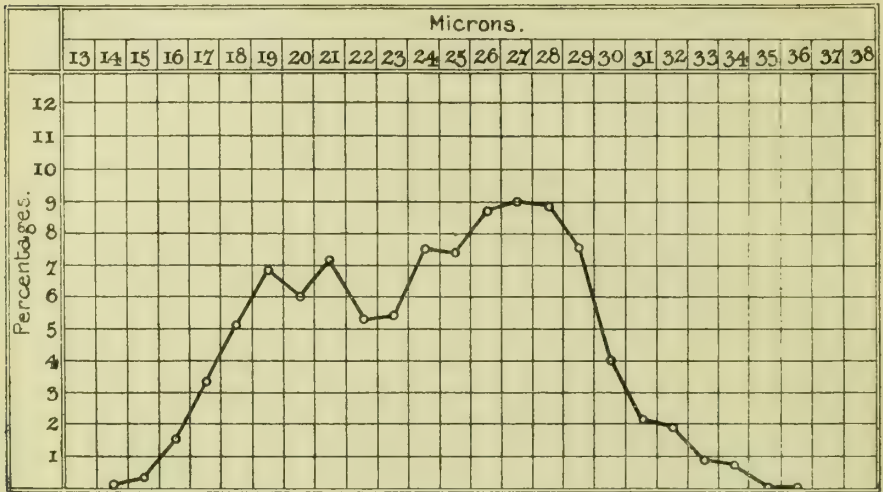
Table II.

Species of animal.	No. of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Man	60	23·9	30·0	18·0
Monkey*	100	20·6	31·0	16·0
Goat	20	24·3	28·0	21·0
Sheep	60	24·9	29·0	22·0
Dog	260	24·2	34·0	15·0
Guinea-pig	120	22·6	36·0	14·0
Rat	600	25·1	35·0	16·0

* Probably *Cercopithecus pygerythrus*, the common grey, black-faced monkey; native name "Pusi."

[illegible]

CHART 1.—Chart giving Curve representing the Distribution, by Percentages, in respect to Length, of 1 220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.



This curve is made up of measurements from 60 specimens of trypanosomes taken from man, 100 from the monkey, 20 from the goat, 60 from the sheep, 260 from the dog, 120 from the guinea-pig, and 600 from the rat.

Table IV.—Measurements giving the Average Distance from the Posterior Extremity to Micronucleus, Micronucleus to Nucleus, etc., in 1,220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.

Posterior extremity to micronucleus.	Micronucleus to nucleus.	Diameter of nucleus.	Nucleus to anterior extremity.	Flagellum.
Short and stumpy (14 to 21 microns), 620 individuals.				
1.4	4.4	2.7	10.0	0.6
Intermediate (22 to 24 microns), 224 individuals.				
1.6	5.6	2.9	9.3	3.7
Long and slender (25 to 36 microns), 376 individuals.				
2.0	6.3	3.1	10.4	5.8

The measurements are made along the curve of the long axis of the body of the trypanosome, and therefore if an oval nucleus is lying transversely to the long axis of the trypanosome, the measurement given will not represent the greatest length of the nucleus.

Breadth.—The long and slender average 1.7 microns in breadth, the intermediate 2.1, and the short and stumpy 2.9. This measurement was made across the broadest part in 100 of each of the three varieties of this trypanosome.

Shape.—The long and slender are very similar in appearance to *T. brucei* and *T. gambiense*, but the intermediate differ to some extent in that the posterior extremity seems often to be more elongated than in the other two species, and blunter (Plate 1, figs. 1, 2, and 3; Plate 2, figs. 6, 8, and 9). The short and stumpy are also characterised by blunter posterior extremities, often suggesting the appearance of a hippopotamus head (Plate 1, figs. 8 and 9). On comparing these plates with those of *T. brucei* and *T. gambiense* this difference will readily be seen.

Contents of Cell.—In well-stained films the protoplasm of many of the trypanosomes shows granules, especially in the anterior portion of the cell.

Table V.—Percentage of Posterior Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human Trypanosome Disease of Nyasaland.

Date.	Experiment No.	Animal.	Percentage of short and stumpy forms.	Proportion to all forms per 1000.
1912.				
Mar. 1	233	Monkey	7	17
" 4	233	"	10	25
" 14	233	"	6	15
Feb. 23	234	"	21	52
Mar. 1	234	"	1	2
Feb. 29	14	Dog	7	17
" 26	157	"	16	40
" 29	243	"	41	102
" 29	13	Guinea-pig	6	15
Mar. 18	13	"	8	20
" 14	165	"	7	17
" 18	165	"	36	90
Feb. 29	166	"	17	42
Mar. 18	239	"	36	90
Feb. 22	235	Rat	7	17
" 23	235	"	22	55
" 27	235	"	38	95
" 28	235	"	41	102
" 29	235	"	39	97
Mar. 1	235	"	51	127
" 4	235	"	29	72
" 5	235	"	30	75
" 6	235	"	36	90
" 7	235	"	30	75
" 8	235	"	38	95
" 11	235	"	39	97
" 13	235	"	45	112
Feb. 27	236	"	24	60
" 29	236	"	32	80
Mar. 1	236	"	46	115

Nucleus.—The nucleus is oval in the long and slender and intermediate, and round or oval in the short and stumpy. One peculiarity about the nucleus of this species is that it is frequently placed far back in the body of the organism. This peculiarity is found in the short and stumpy non-flagellar forms (Plate 1, figs. 4 to 11) and more rarely in the intermediate

(Plate 1, figs. 12 and 13); never in the long and slender. The percentage among the short and stumpy forms is often large, as will be seen by Table V. As the percentage of these non-flagellar forms is 24·8 of the whole, it is easy to calculate the percentage of the posterior nuclear forms to the whole body of this trypanosome.

Micronucleus.—Small and round, and situated, on an average, 2 microns from the posterior extremity in the long and slender, 1·6 in the intermediate, and 1·4 in the short and stumpy.

Undulating Membrane.—This, as in *T. brucei* and *T. gambiense*, is well developed and thrown into bold folds and undulations.

Flagellum.—The flagellum in the long and slender averages 5·8 microns (maximum 11, minimum 2), and in the intermediate 3·3 microns (maximum 9, minimum 1). There is no free flagellum in the short and stumpy forms. In *T. brucei* the short and stumpy, non-flagellated forms mostly lie between 13 and 21 microns. This is not so in the trypanosome under consideration, as the following table shows:—

Table VI.—Number of Flagellated and Non-flagellated Forms found among 1,220 Trypanosomes of the Human Trypanosome Disease of Nyasaland.

Length, mi- crons.	Non- flagel- lated.	Flagel- lated.	Length, mi- crons.	Non- flagel- lated.	Flagel- lated.	Length, mi- crons.	Non- flagel- lated.	Flagel- lated.
Short and stumpy.			Intermediate.			Long and slender.		
14	1	—	22	5	60	25	—	91
15	4	—	23	2	64	26	—	107
16	19	—	24	—	93	27	—	110
17	42	—				28	—	104
18	62	1				29	—	87
19	74	7				30	—	49
20	56	19				31	—	27
21	37	54				32	—	23
						33	—	13
						34	—	7
						35	—	1
						36	—	1
295			7			0		
81			217			620		

The above table shows that 81 of the intermediate forms are under 22 microns in length, and that seven short and stumpy forms are above 21 in length. If the trypanosomes are separated into non-flagellar and flagellated, then there are 24·8 per cent. of the former and 75·2 per cent. of the latter.

COMPARISON OF THE TRYPANOSOME OF THE HUMAN TRYPANOSOME DISEASE OF NYASALAND WITH *T. BRUCEI*.

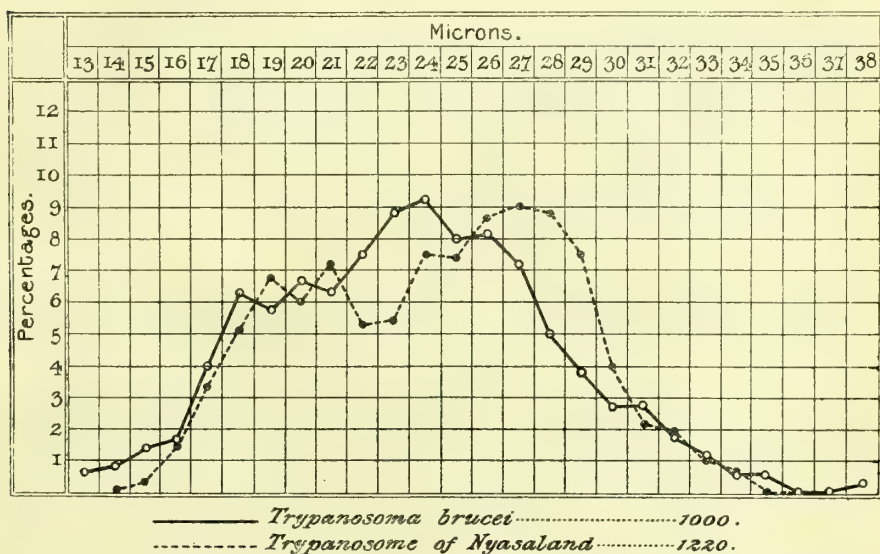
The coloured plate which accompanies this paper, when compared with that of *T. brucei** or *T. gambiense*,† shows that the

* 'Roy. Soc. Proc.,' 1911, B, vol. 83, Plate 2. (S.S. Reports No. XI., Pl. 5.)

† *Ibid.*, vol. 84, Plate 13. (S.S. Reports No. XII., Pl. 2.)

three species are much alike. The chief difference is the tendency of the Nyasaland trypanosome, as has already been pointed out, to show numerous posterior nuclear forms. The Commission has had no opportunity of examining *T. brucei* for posterior nuclear forms under the same conditions as the Nyasaland organism, but it may be assumed that *T. brucei* does not show these aberrant forms to the same extent, or the fact would have been noted long ago, no species having been studied so often or so closely as *T. brucei*. Another difference between the two species is the occurrence of the broad, blunt-ended forms—the hippo-headed—among the short and stumpy, a form which rarely, if ever, appears in Bruce's trypanosome.

CHART 2.—Chart giving Curves representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of *T. brucei*, and 1220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.



But when we compare the curves representing the distribution by percentages in respect to length of 1,000 and 1,220 individuals of each species, the similarity of the two parasites is remarkable. In *T. brucei* there are 26 per cent. non-flagellated forms, in the trypanosome of Nyasaland 25 per cent.

Again, if we divide the 1,220 Nyasaland trypanosomes, by length, into short and stumpy (13-21 microns), intermediate (22-24 microns), and long and slender (25 microns and upwards), as has been done in the case of *T. brucei* and *T. gambiense*, the result is as follows:—

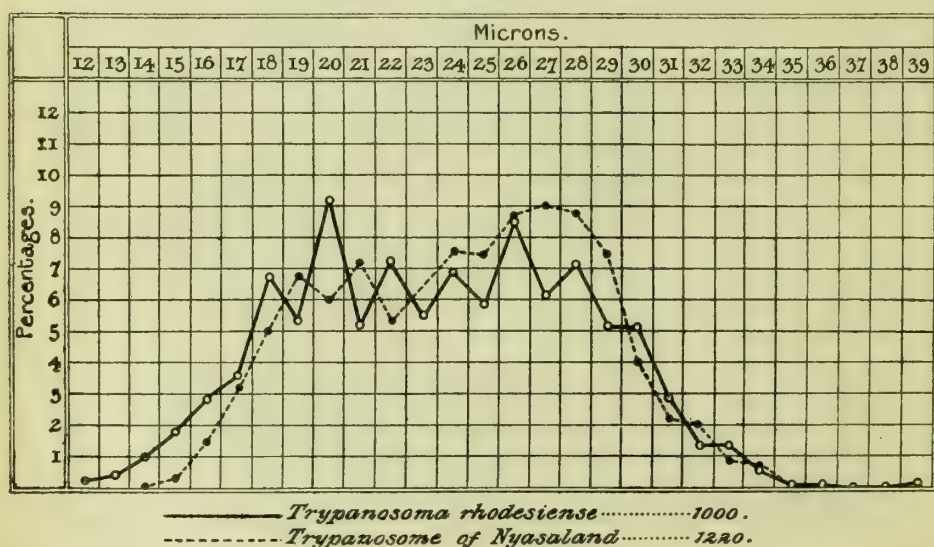
	Short and stumpy.	Intermediate.	Long and slender.
<i>T. gambiense</i>	51·2	23·1	25·7
<i>T. brucei</i>	32·8	25·5	41·7
Nyasaland trypanosome	30·8	18·4	50·8

This shows the percentage of the short and stumpy to be almost the same in *T. brucei* and the Nyasaland trypanosome, and the latter to be rather better off in the long and slender forms.

COMPARISON OF THE TRYPANOSOME OF THE HUMAN TRYPANOSOME
DISEASE OF NYASALAND WITH *T. RHODESIENSE* (STEPHENS
AND FANTHAM).

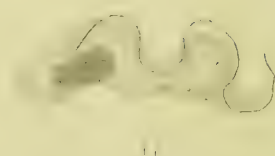
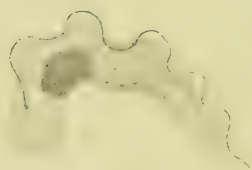
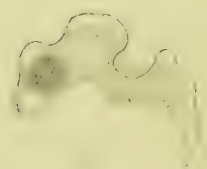
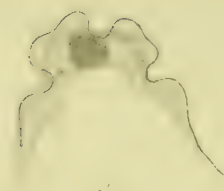
Dr. J. W. W. Stephens, of the Liverpool School of Tropical Medicine, at Sir David Bruce's request, kindly measured 1,000 trypanosomes of the strain from a case of Sleeping Sickness from the Luangwa Valley, North-east Rhodesia, which he has named *T. rhodesiense*. This proposed new species is characterised by posterior nuclear forms, and a snout-like prolongation of the posterior extremity. These are also, as we have seen, characteristics of the Nyasaland trypanosome. The following chart has been prepared from the figures supplied by Dr. Stephens, and shows that the similarity of the curve prepared from 1,000 individuals of *T. rhodesiense* to that prepared from 1,220 individuals of the Nyasaland trypanosome is still more remarkable than in the case of *T. brucei*. Dr. Stephens measured 100 trypanosomes from man, 40 from the monkey, 40 from the horse, 40 from the dog, 40 from the rabbit, 100 from the guinea-pig, 600 from the rat, and 40 from the mouse.

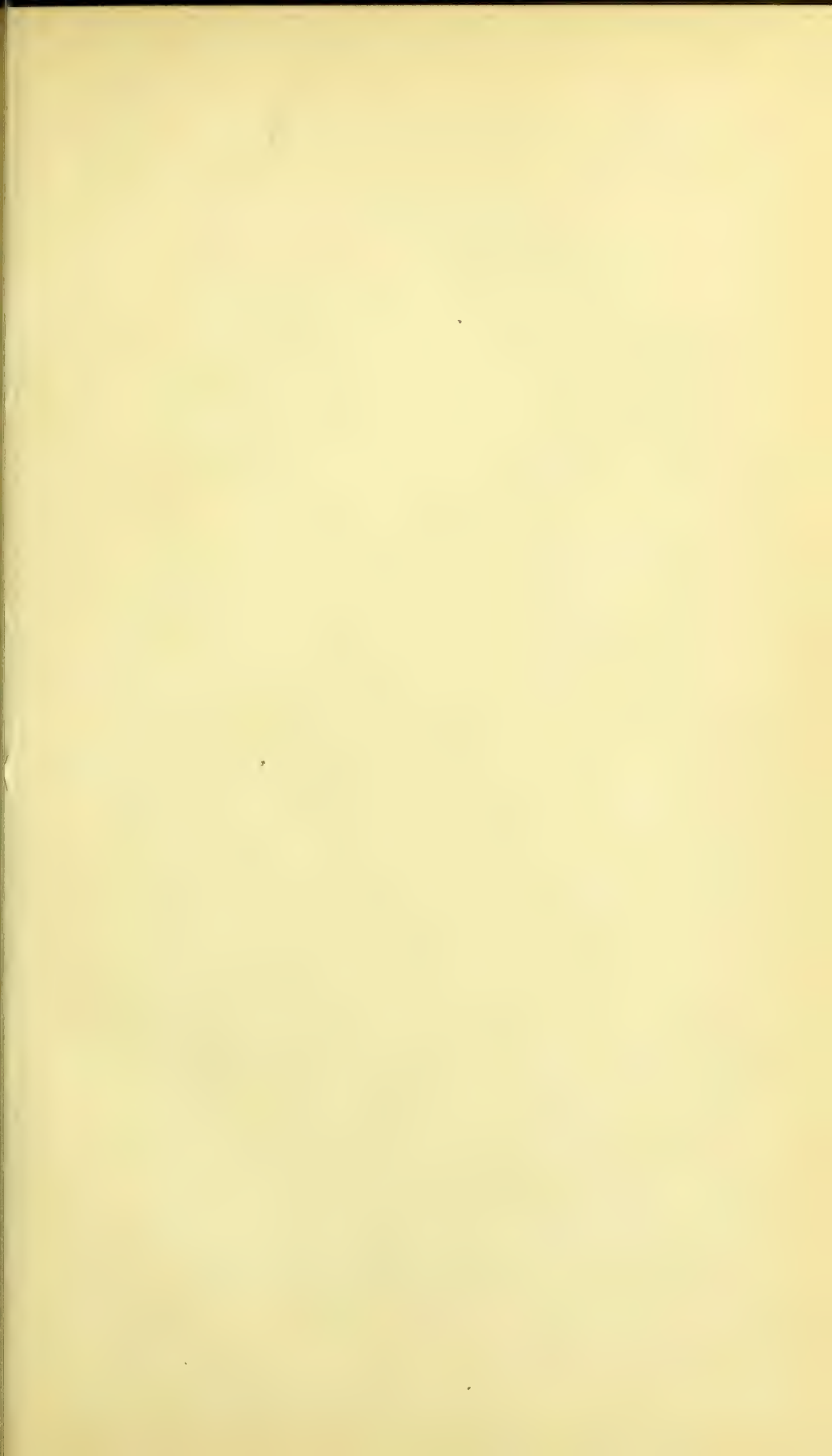
CHART 3.—Chart giving Curves representing the Distribution, by Percentages, in respect to Length, of 1,000 Individuals of *T. rhodesiense*, and 1,220 Individuals of the Trypanosome of the Human Trypanosome Disease of Nyasaland.

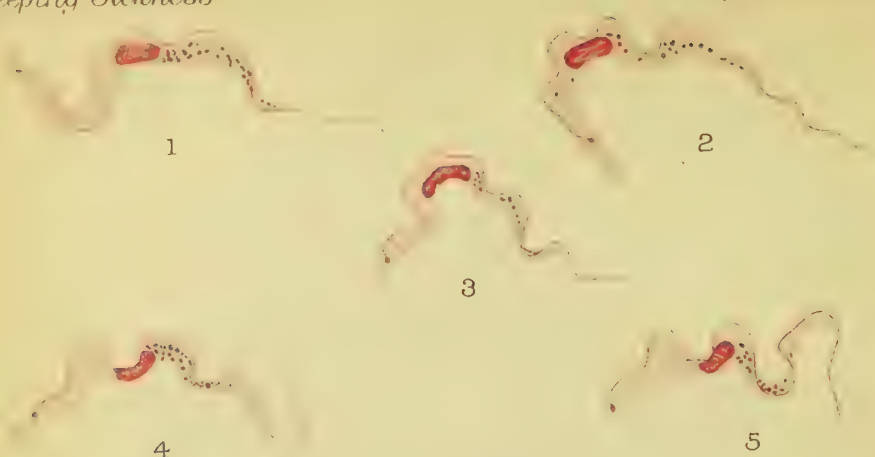


Conclusions.

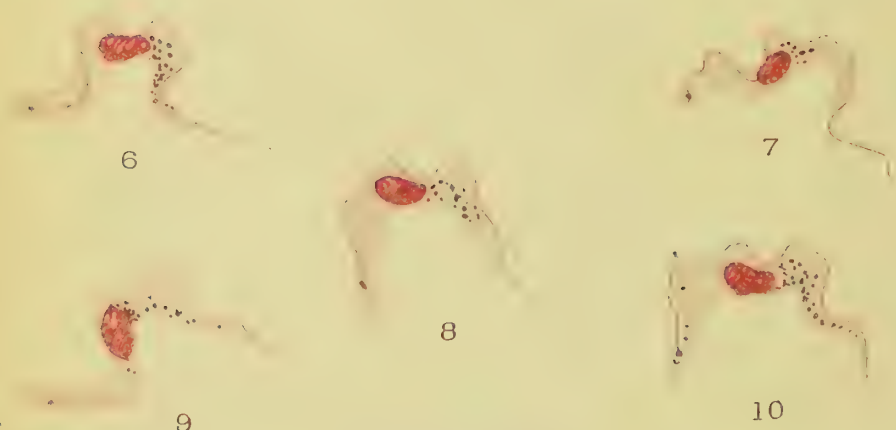
1. The trypanosome of the human trypanosome disease of Nyasaland is *T. rhodesiense* (Stephens and Fantham).
2. This is a distinct species, nearly related to *T. brucei* and *T. gambiense*, but more closely resembling the former than the latter.



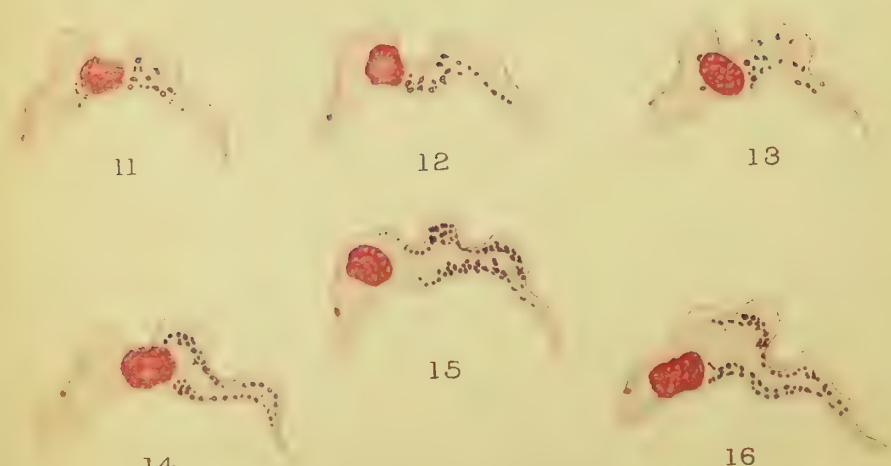




Long & Slender



Intermediate



Short & Stumpy

3. From this it follows that the human trypanosome disease of North-east Rhodesia and Nyasaland is not the disease known as Sleeping Sickness in Uganda and the West Coast of Africa.

4. The native name "Kaodzera" might be used for this new disease to distinguish it from the older known Sleeping Sickness

DESCRIPTION OF PLATES.

PLATE 1.

Trypanosoma rhodesiense, posterior nuclear and blunt, hippo-headed forms found commonly in this species. $\times 2,000$.

Figs. 1, 2, and 3. Intermediate forms with blunt posterior extremities.

Figs. 4, 5, 7, 8, 9, 10, and 11. Posterior nuclear forms.

Fig. 6. Nucleus near anterior extremity.

Figs. 12 and 13. Posterior nuclear intermediate forms.

PLATE 2.

Trypanosoma rhodesiense, Nyasaland, 1912, stained Giemsa. $\times 2,000$.

Figs. 1-5. Long and slender forms.

Figs. 6-10. Intermediate forms.

Figs. 11-16. Short and stumpy forms.

II.—THE MORPHOLOGY OF *TRYPANOSOMA SIMIÆ*, SP. NOV.*

By Colonel SIR DAVID BRUCE, C.B., F.R.S., A.M.S.; Majors DAVID HARVEY, and A. E. HAMERTON, D.S.O., R.A.M.C.; Dr. J. B. DAVEY, Nyasaland Medical Staff; and Lady BRUCE, R.R.C.

(Received August 3, 1912.)

[Plate 3.]

INTRODUCTION.

This species of trypanosome, which does not seem to have been described before, is remarkable in that it attacks only such widely different animals as the monkey and the goat. Oxen, baboons, dogs, guinea-pigs, and white rats appear to be immune. The rapidity with which it kills monkeys is very striking. In a series of 19 the average duration of life after the trypanosomes were first seen in the blood was only 2·9 days. Its action on animals, its reservoir, its carrier, and cultivation, have not been fully worked out, and will form the subject of a future paper.

* Reprinted from Proceedings of the Royal Society, Series B, vol. 85, pp. 477, &c.

In regard to its carrier, it may be stated that in this district it is *Glossina morsitans*, and that scarcely a single cage of flies is brought to Kasu Hill from the neighbouring "fly-country" but is found to be infected with this trypanosome.

A. Living, Unstained.

Trypanosoma simiæ shows active translatory movements when alive: some individuals pass completely across the field of the microscope. Apparently the usual mode of progression is flagellum first, but occasionally an individual can be seen to move a short distance in the opposite direction.

B. Fixed and Stained.

The blood films were fixed, stained, and measured, as previously described in the "Proceedings."*

Length.—The following table gives the length of this trypanosome as found in the monkey and the goat, 500 trypanosomes in all.

Table I.—Measurements of the Length of *Trypanosoma simiæ*.

Date.	No. of Ex- peri- ment.	Animal.	Method of fixing.	Method of staining.	In microns.			
					Average length.	Maximum length.	Minimum length.	
1912.								
Feb. 12 ...	117	Goat	Osmic acid	Giemsa	17·2	18·0	15·0	
" 15 ...	117	"	"	"	16·8	18·0	15·0	
" 22 ...	109	"	"	"	16·7	18·0	15·0	
Mar. 11 ...	117	"	"	"	16·5	19·0	14·0	
" 18 ...	247	"	"	"	18·2	21·0	15·0	
June 13 ...	620	"	"	"	18·4	21·0	17·0	
" 13 ...	620	"	"	"	17·4	20·0	15·0	
Jan. 29 ...	20	Monkey	"	"	17·6	19·0	16·0	
Feb. 1 ...	20		"	"	17·5	20·0	15·0	
" 6 ...	54		"	"	18·9	22·0	16·0	
" 6 ...	55		"	"	17·5	20·0	15·0	
" 8 ...	54		"	"	18·5	20·0	16·0	
" 12 ...	49		"	"	18·4	21·0	15·0	
" 12 ...	54		"	"	18·7	24·0	17·0	
" 12 ...	59		"	"	17·5	19·0	15·0	
" 23 ...	58		"	"	16·0	18·0	14·0	
" 27 ...	58		"	"	17·4	19·0	16·0	
Mar. 18 ...	286	"	"	"	16·1	18·0	14·0	
Apr. 4 ...	58	"	"	"	18·6	20·0	15·0	
" 8 ...	286	"	"	"	17·2	20·0	15·0	
" 18 ...	404	"	"	"	19·8	22·0	16·0	
" 18 ...	448	"	"	"	20·4	23·0	18·0	
" 23 ...	405	"	"	"	19·0	21·0	17·0	
" 25 ...	449	"	"	"	18·6	21·0	16·0	
" 29 ...	448	"	"	"	18·9	21·0	16·0	
					17·5	24·0	14·0	

* 'Roy. Soc. Proc.' 1909, B, vol. 81, pp. 16 and 17. (S.S. Reports No. X., p. 12.)

The average length of *T. simiæ* in the monkey and goat, taken from Table I, is as follows:—

Table II.—Average Length of *T. simiæ*.

Species of animal.	No. of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Monkey	360	18·1	24·0	14·0
Goat	140	17·2	21·0	14·0

Table III.—Distribution in respect to Length of 500 Individuals of *T. simiæ*.

Animal.			In microns.										Average length.
			14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	
Goat	...	...	—	1	5	5	9	—	—	—	—	—	17·2
"	...	...	—	3	8	7	2	—	—	—	—	—	16·8
"	...	...	1	2	5	8	2	2	—	—	—	—	16·7
"	...	...	2	1	10	1	3	3	—	—	—	—	16·5
"	...	...	—	1	2	3	5	5	2	2	—	—	18·2
"	...	...	—	—	—	3	9	6	1	1	—	—	18·4
"	...	...	—	2	2	7	4	4	1	—	—	—	17·4
Monkey	...	...	—	—	4	7	8	1	—	—	—	—	17·6
"	...	...	—	1	2	7	6	3	1	—	—	—	17·5
"	...	...	—	—	1	3	6	2	4	3	1	—	18·9
"	...	...	—	2	3	8	3	3	1	—	—	—	17·5
"	...	...	—	—	1	3	5	6	5	—	—	—	18·5
"	...	...	—	2	1	2	5	7	2	1	—	—	18·4
"	...	...	—	—	—	3	8	4	4	—	—	1	18·7
"	...	...	—	1	6	4	5	4	—	—	—	—	17·5
"	...	...	2	5	7	2	4	—	—	—	—	—	16·0
"	...	...	—	—	4	6	8	2	—	—	—	—	17·4
"	...	...	2	4	6	5	3	—	—	—	—	—	16·1
"	...	...	—	1	1	—	5	9	4	—	—	—	18·6
"	...	...	—	2	5	4	6	2	1	—	—	—	17·2
"	...	...	—	—	1	1	2	4	4	5	3	—	19·8
"	...	...	—	—	—	—	2	3	6	5	2	2	20·4
"	...	...	—	—	—	1	5	8	5	1	—	—	19·0
"	...	...	—	—	1	3	5	7	2	2	—	—	18·6
"	...	...	—	—	1	—	6	7	4	2	—	—	18·9
Total	...	...	7	28	76	93	126	92	47	22	6	2	1
Percentages	1	4	5·6	15·2	18·6	25·2	18·4	9·4	4·4	1·2	0·4	0·2	

CHART 1.—Chart giving Curve representing the Distribution, by Percentages, in respect to Length of 500 Individuals of *T. simiæ*



From this curve it will be seen that *T. simiæ* is a monomorphic species, varying from 14 to 24 microns in length, the greatest number of individuals (25.2 per cent.) being 18 microns long.

Breadth.—Measured across the broadest part *T. simiæ* averages 1.75 microns in breadth (maximum 2.75, minimum 1).

Shape.—These trypanosomes are monomorphic and, as a rule, fairly uniform in shape. The body is elongated, markedly undulating, and frequently extends in a straight line (Plate 3, fig. 2). The posterior extremity is bluntly pointed or rounded, and there is frequently the appearance of a vacuole at the extreme end, as shown in figs. 6, 9, 11, 16, 17, and 18. The anterior extremity is pointed.

Contents of Cell.—Clear, homogeneous, and free from granules.

Nucleus.—Oval, and situated about the middle of the body.

Micronucleus.—Small and round, situated almost invariably about $1\frac{1}{2}$ microns from the posterior extremity. A peculiarity is that it is almost always placed at the edge of the trypanosome, from which it seems to protrude, or to be on the point of falling out. This is so marked that in the laboratory this trypanosome became known as the “glad eye,” from the well-known play of this name.

Undulating membrane.—Well developed and thrown into bold undulations, herein differing from *T. vivax* and *T. uniforme*.

Flagellum.—It is difficult to say whether this species has a free flagellum or not. By careful staining and good illumination it would seem in most cases as if the undulating membrane extended to the tip of the flagellum. In most preparations, however, the last two or three microns of the flagellum often appear to be free. This is shown in Plate 3.

Division Forms.—In the monkey, in which these trypanosomes swarm in enormous numbers, masses of them can be seen, sometimes filling up the whole field of the microscope. It would seem as if multiplication took place so rapidly that the individual





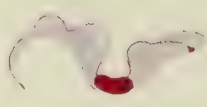
1



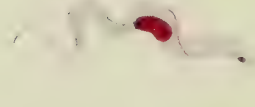
2



3



4



5



6



7



8



9



10

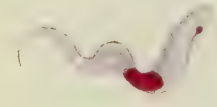
Monkey.



11



12



13



14



15



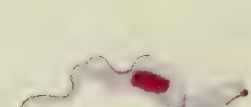
16



17



18



19



20

Goat.

trypanosomes had not time to disengage themselves. A small part of such mass is represented in fig. 1 in the text.

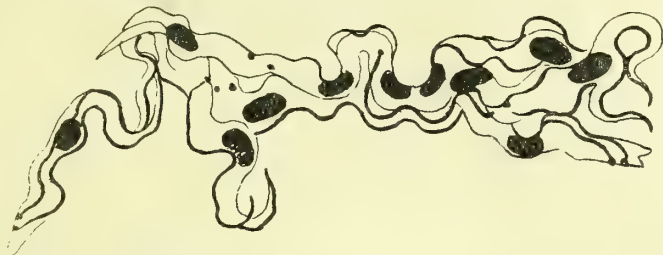


FIG. 1.

In addition to this, numerous division forms are seen, often four or five in a field, in which the trypanosomes appear to slip past one another until they are only joined by their non-flagellar ends, as shown in fig. 2.



FIG. 2.

Conclusions.

1. *T. simiæ*, sp. nov., is a well-defined species, easily separated by its morphology alone from the other trypanosomes which have been described as causing disease among domestic animals.

2. It sets up a chronic disease in goats, but is chiefly remarkable for its rapidly fatal action on monkeys.

3. In Nyasaland it is carried by *G. morsitans*, and in this district—Central Angoniland—this tsetse-fly is found to be heavily infected with this trypanosome.

DESCRIPTION OF PLATE 3.

Trypanosoma simiæ, sp. nov.—Elongated, narrow, undulating body; posterior extremity bluntly pointed or rounded; anterior extremity pointed; nucleus oval; micronucleus small, round, situated about 1.5 microns from posterior extremity, placed laterally, protuberant; undulating membrane marked, thrown into bold folds; flagellum frequently not projecting beyond undulating membrane, sometimes 1 or 2 microns of the extremity apparently free.

Figs. 1-10, *T. simiæ* from the monkey; figs. 11-20 from the goat. Stained Giemsa. $\times 2,000$.

III.—THE TRYPANOSOMES FOUND IN THE BLOOD OF WILD ANIMALS LIVING IN THE SLEEPING-SICKNESS AREA, NYASALAND.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Majors DAVID HARVEY and A. E. HAMERTON, D.S.O., R.A.M.C.; Dr. J. B. DAVFY, Nyasaland Medical Staff;† and Lady BRUCE, R.R.C.

(Received January 13, 1913.)

INTRODUCTION.

The chief object of this Commission in coming to Nyasaland was to inquire into the relation of the African fauna to the maintenance and spread of trypanosome disease.

The Commission arrived at their camp on Kasu Hill on January 12, 1912. As this was the rainy season the low country was covered with dense vegetation and much of it under water. Nothing could therefore be done in the study of the fauna until about the beginning of June, when the dry season was well established.

The camp at Kasu is situated on one of the hills (lat. $13^{\circ} 40'$ S., long. $34^{\circ} 12'$ E.) which rise on the western edge of the flat country adjoining Lake Nyasa. This low-lying lake-coast plain looks quite flat when viewed from the camp, and extends from the lake shore some 20 miles inland. The camp lies about 10 miles from the edge of this low country, and, therefore, some 30 miles from the lake. This plain is covered with thorn scrub, except near the lake, where there are large grassy plains, or "dambos," dotted over with palm trees. The thorn-scrub is the home of the tsetse-fly and also of numerous wild animals.

When an animal is shot in this fly-country by a member of the Commission a small quantity of the blood is taken in a bottle containing citrate of soda solution for inoculation purposes, and a thick and thin film of the blood spread on glass slides for microscopical examination. The blood is then sent to a point on one of the main paths, where a motor-cyclist is waiting to carry it to the camp. When the blood arrives it is at once injected into a goat, a monkey, and a dog.

WILD ANIMALS LIVING IN A FLY-AREA AS HOSTS OR RESERVOIRS OF TRYPANOSOME DISEASE.

The following table represents the result of the examination of 180 specimens of wild game or other animals shot in this fly-area. In one column is given the number of hours between the taking of the blood and its inoculation, in others the result of microscopical examination of the thick and thin film, and of the inoculation of the blood. A plus sign means that trypanosomes are present, a minus sign that they are absent, and a blank space that a microscopical specimen was not available for examination, or an animal for inoculation, as the case may be.

* Reprinted from the Proceedings of the Royal Society, B. Vol. 86.

† Dr. Davey resigned his membership of the Commission in October, 1912, before the completion of the work here recorded.

Table I.—List of Wild Animals living in a Fly-area whose Blood has been Examined for Trypanosomes.

Date.	Experi- ment No.	Animal.	Age of blood, in hours.	By microscopical examination.		By inoculation.			
				Thick.	Thin.	Goat.	Monkey.	Dog.	
1912.									
Jan. 20 ...	32	Hartebeeste		—	—	—		—	
" 20 ...	33	"			—	—		—	
" 20 ...	34	"			—	—		—	
" 22 ...	40	"			—	—		—	
" 22 ...	41	Eland ...			—	—		+	
" 22 ...	44	" ...			—	+		—	
" 23 ...	62	Sable ...		—	—	—		—	
" 23 ...	63	" ...			—	—		—	
" 26 ...	95	Eland ...		—	—	—		—	
Feb. 6 ...	159	Warthog ...			—	—		—	
" 6 ...	160	Sable ...			—	—		—	
" 18 ...	246	Elephant ...			—	—		—	
" 23 ...	283	Eland ...			—	—		—	
" 28 ...	288	Lion ...			—	—		—	
May 19 ...	615	Oribi ...	3	—	—	—		—	
" 19 ...	616	Waterbuck	17		+	—		—	
" 25 ...	583	Duiker ...			—	—	—	—	
" 25 ...	584	Warthog ...			—	—	—	—	
" 26 ...	589	" ...			—	—		—	
" 26 ...	591	Reedbuck...			+	—		—	
" 26 ...	593	Duiker ...			—	—		—	
June 2 ...	611	Buffalo ...	9	—	—	—	—	—	
" 2 ...	612	" ...	9	—	—	—	—	—	
" 2 ...	613	" ...	9	+	—	—	—	—	
" 2 ...	614	" ...	9	—	—	—	—	—	
" 15 ...	686	Warthog ...	9½	—	—	—	—	—	
" 15 ...	687	Oribi ...	9½	—	—	—	—	—	
" 16 ...	692	Reedbuck...	8½	—	—	—	—	—	
" 16 ...	695	Sable ...	5	—	—	—	—	—	
" 23 ...	742	Warthog ...	9	—	—	—	—	—	
" 23 ...	742 _a	" ...	9	—	—	—	—	—	
" 23 ...	743	Oribi ...	9	+	—	—	—	—	
" 23 ...	743 _a	" ...	9	—	—	—	—	—	
" 25 ...	744	Duiker ...	7½	—	—	—	—	—	
" 27 ...	755	Bushbuck...	11	—	—	—	—	—	
" 29 ...	768	Hartebeeste	6½	—	—	—	—	—	
" 29 ...	769	Reedbuck...	5½	—	—	—	—	—	
" 29 ...	780	" ...	2	—	—	—	—	—	
" 30 ...	783	" ...	$\frac{1}{4}$	+	+	—	+	+	
July 1 ...	777	Hartebeeste	7			—	—	—	
" 1 ..	778	"	7			—	—	—	
" 1 ...	779	"	7			+	+	+	
" 2 ...	820	Oribi ...	7½		—	—	—	—	
" 3 ...	801	" ...	7½	—	—	—	—	—	
" 3 ...	818	Warthog ...	11¾	—	—	—	—	—	
" 4 ...	813	Hartebeeste	8	—	—	—	—	—	
" 4 ...	814	"	8	—	—	—	—	—	
" 4 ...	815	"	8	—	—	—	—	—	
" 4 ...	816	"	8	—	—	—	—	—	
" 4 ...	817	"	8	—	—	—	—	—	
" 5 ...	828	Reedbuck...		+	—	—	—	—	

Table I—continued.

Date.	Experiment No.	Animal.	Age of blood, in hours.	By microscopical examination.		By inoculation.		
				Thick.	Thin.	Goat.	Monkey.	Dog.
1912.								
July 6 ...	825	Hartebeeste	8	—	—			
" 6 ...	826	Warthog ...	$7\frac{3}{4}$	+	+	—	—	—
" 6 ...	827	Oribi ...	$7\frac{1}{4}$	—	—	—	—	—
" 8 ...	844	Hartebeeste	$4\frac{3}{4}$	—	—	—	—	—
" 8 ...	863	Oribi ...	$1\frac{1}{4}$	—	—		+	—
" 10 ...	859	Hartebeeste	$9\frac{3}{4}$	—	—	—	—	—
" 10 ...	860	Oribi ...	$5\frac{3}{4}$	—	+	—	—	—
" 10 ...	861	Warthog ...	$6\frac{3}{4}$	—	—	—	—	—
" 10 ...	862	Oribi ...	$5\frac{3}{4}$	—	—	—	—	—
" 11 ...	866	" ...	$7\frac{1}{2}$	—	—	—	—	—
" 11 ...	869	Warthog ...	$8\frac{1}{2}$	—	—	—	—	—
" 11 ...	872	" ...	$7\frac{1}{2}$	—	—	—	—	—
" 11 ...	875	Hartebeeste	$5\frac{3}{4}$	—	—	—	—	—
" 20 ...	912	Reedbuck...	$7\frac{1}{4}$	—	—	+	—	—
" 21 ...	918	Hartebeeste	8	—	—	—	—	—
" 21 ...	919	Oribi ...	7	—	—	—	—	—
" 21 ...	920	Warthog ...	5	—	—	—	—	—
" 21 ...	920 _a	" ...	5	—	—	—	—	—
" 21 ...	920 _b	" ...	5	—	—	—	—	—
" 21 ...	921	Oribi ...	8	—	—	—	—	—
" 21 ...	923	Warthog ...	10	—	—	—	—	—
" 21 ...	925	Bushbuck...	10	—	—	—	—	—
" 22 ...	927	Hartebeeste	$7\frac{3}{4}$	—	—	—	—	—
" 22 ...	929	Warthog ...	$5\frac{3}{4}$	—	—	—	—	—
" 22 ...	931	" ...	$5\frac{1}{4}$	—	—	—	—	—
" 22 ...	933	" ...	$4\frac{1}{2}$	+	+	—	—	—
" 22 ...	935	" ...	$4\frac{1}{2}$	—	—	—	—	—
" 23 ...	955	Hyæna ...	8	—	—	+	—	—
" 23 ...	956	" ...	8	+	—	—	—	—
" 23 ...	957	Hartebeeste	5	—	—	+	+	+
" 23 ...	958	" ...	$3\frac{1}{2}$	+	+	—	—	—
" 25 ...	988	Reedbuck...	$6\frac{3}{4}$	+	+	+	—	—
" 26 ...	993	Duiker ...	$5\frac{1}{2}$	—	—	—	—	—
" 27 ...	1000	Hartebeeste	$3\frac{1}{2}$	+	—	+	+	+
" 29 ...	1004	" ...	$5\frac{1}{4}$	—	—	—	—	—
" 29 ...	1007	Duiker ...	4	+	—	—	—	—
" 30 ...	1010	Hartebeeste	4	—	—	—	—	—
" 30 ...	1013	Eland ...	4	+	+	+	+	+
Aug. 1 ...	1017	Oribi ...	$8\frac{1}{4}$	—	—	—	—	—
" 2 ...	1024	Sable ...	$7\frac{1}{4}$	—	—	—	—	—
" 2 ...	1027	Duiker ...	$5\frac{1}{4}$	+	—	—	—	—
" 4 ...	1044	Eland ...	$6\frac{1}{2}$	—	—	+	—	—
" 5 ...	1045	Duiker ...	$6\frac{3}{4}$	—	—	—	—	—
" 5 ...	1048	Wild cat ...	3	—	—	—	—	—
" 7 ...	1052	Warthog ...	$5\frac{3}{4}$	—	—	—	—	—
" 7 ...	1055	Wild cat ...	$2\frac{3}{4}$	—	—	—	—	—
" 11 ...	1058	Koodoo ...	$3\frac{1}{2}$	+	+	—	—	—
" 11 ...	1061	Waterbuck..	$2\frac{3}{4}$	+	—	+	—	—
" 11 ...	1064	Warthog ...	$2\frac{3}{4}$	+	+	—	—	—
" 12 ...	1067	Hyæna ...	4	—	—	—	—	—
" 18 ...	1075	Waterbuck..	5	—	—	—	—	—
" 18 ...	1078	Bushbuck...	3	+	+	+	—	—
" 18 ...	1081	" ...	3	+	—	—	—	—

Table I.—continued.

Date.	Experiment No.	Animal.	Age of blood, in hours.	By microscopical examination.		By inoculation.		
				Thick.	Thin.	Goat.	Monkey.	Dog.
1912.								
Aug. 18 ...	1084	Bushbuck...	3	+	+	+	+	+
" 18 ...	1087	" ...	3	+	+	+	—	—
" 19 ...	1090	Oribi ...	7	—	—	—	—	—
" 19 ...	1093	" ...	7	—	—	—	—	—
" 19 ...	1096	" ...	7	—	—	+	—	—
" 19 ...	1099	" ...	7	—	—	—	—	—
" 19 ...	1102	" ...	7	—	—	—	—	—
" 21 ...	1136	Warthog ...	5 $\frac{3}{4}$	—	—	—	—	—
" 21 ...	1139	" ...	5	+	+	+	—	—
" 21 ...	1142	Hartebeeste	7 $\frac{1}{2}$	—	—	+	—	+
" 21 ...	1145	" ...	6 $\frac{3}{4}$	—	—	—	—	—
" 22 ...	1150	Reedbuck...	8 $\frac{1}{2}$	+	+	+	—	—
" 22 ...	1153	" ...	8 $\frac{1}{2}$	+	—	+	—	—
" 22 ...	1156	" ...	7	+	—	+	—	—
" 22 ...	1159	" ...	7	—	—	—	—	—
" 22 ...	1162	" ...	6	+	—	+	—	—
" 22 ...	1165	" ...	6	—	—	—	—	—
" 23 ...	1168	Warthog ...	7 $\frac{3}{4}$	—	—	—	—	—
" 23 ...	1171	Wild cat ...	6 $\frac{3}{4}$	—	—	—	—	—
" 23 ...	1174	Waterbuck..	7	—	—	—	—	—
" 24 ...	1177	" ...	5	—	—	—	—	—
" 24 ...	1180	" ...	5	+	—	+	+	+
" 24 ...	1183	Warthog ...	7 $\frac{1}{2}$	—	—	—	—	—
" 24 ...	1186	" ...	6	+	—	+	—	—
" 24 ...	1189	" ...	6	+	—	—	—	—
" 24 ...	1192	Oribi ...	7 $\frac{1}{4}$	—	—	—	—	—
" 24 ...	1195	" ...	7 $\frac{1}{4}$	—	—	—	—	—
" 24 ...	1198	Porcupine ..	8 $\frac{1}{2}$	—	—	—	—	—
" 28 ...	1202	Eland ...	4	+	+	+	—	+
" 28 ...	1203	Bushbuck...	5	+	+	—	—	—
" 28 ...	1205	Eland ...	4	—	—	—	—	—
" 28 ...	1210	Waterbuck..	4	+	—	+	+	+
" 30 ...	1216	Bushbuck...	8	+	—	—	—	—
Sept. 6 ...	1250	Koodoo ...	2	—	—	—	—	—
" 6 ...	1254	Oribi ...	6 $\frac{3}{4}$	—	—	—	—	—
" 7 ...	1261	Bushbuck...	4 $\frac{1}{4}$	—	—	+	—	—
" 7 ...	1264	Waterbuck..	3 $\frac{1}{4}$	+	+	—	+	+
" 7 ...	1268	Buffalo ...	6 $\frac{1}{4}$	—	—	—	—	—
" 7 ...	1272	Hartebeeste	5 $\frac{1}{4}$	—	—	—	—	—
" 7 ...	1276	Warthog ...	4	—	—	—	—	—
" 7 ...	1281	Buffalo ...	9	—	—	—	—	—
" 10 ...	1285	" ...	5	—	—	—	—	—
" 10 ...	1289	Eland ...	8 $\frac{1}{4}$	—	—	—	—	—
" 10 ...	1293	Warthog ...	12 $\frac{3}{4}$	—	—	—	—	—
" 10 ...	1298	Buffalo ...	5	—	—	—	—	—
" 11 ...	1304	" ...	3 $\frac{1}{4}$	—	—	+	—	—
" 12 ...	1308	Warthog ...	6	—	—	+	—	—
" 13 ...	1339	Waterbuck..	6 $\frac{1}{2}$	—	—	+	—	—
" 13 ...	1343	Bushbuck...	7	—	—	—	—	—
" 13 ...	1347	Reedbuck...	6	+	—	—	+	+
" 13 ...	1351	" ...	5 $\frac{3}{4}$	—	—	—	—	—
" 14 ...	1355	Hartebeeste	7 $\frac{1}{4}$	—	—	—	—	—
" 14 ...	1359	" ...	7 $\frac{1}{2}$	—	—	—	—	—
" 14 ...	1363	Reedbuck...	6	—	—	+	—	—

Table I.—*continued.*

Date.	Experiment No.	Animal.	Age of blood, in hours.	By microscopical examination.		By inoculation.		
				Thick.	Thin.	Goat.	Monkey.	Dog.
1912.								
Sept. 16 ...	1368	Oribi ...	7 $\frac{1}{4}$	—	—	—	—	—
" 16 ...	1372	" ...	7 $\frac{1}{4}$	—	—	—	—	—
" 16 ...	1376	Elephant ...	20	—	—	—	—	—
" 17 ...	1380	Koodoo ...	2 $\frac{1}{2}$	—	—	+	—	—
" 17 ...	1384	Warthog ...	6 $\frac{1}{2}$	—	—	—	—	—
" 18 ...	1388	Waterbuck..	8	+	+	+	—	—
" 18 ...	1392	Hartebeeste	5	—	—	—	—	—
" 18 ...	1396	"	5	—	—	—	—	—
" 18 ...	1400	Oribi ...	4 $\frac{1}{2}$	—	—	—	—	—
" 20 ...	1406	Waterbuck..	9	—	—	+	—	—
" 20 ...	1410	"	9	—	+	—	—	—
" 20 ...	1414	Warthog ...	6 $\frac{1}{2}$	—	—	—	—	—
" 20 ...	1418	Hartebeeste	—	—	—	—	—	—
" 20 ...	1422	"	9	—	—	—	—	—
" 20 ...	1426	"	7	—	—	—	—	—
" 20 ...	1435	Reedbuck...	9	—	—	—	+	+
" 20 ...	1439	" ...	8 $\frac{1}{2}$	—	—	—	—	—
" 20 ...	1443	Oribi ...	7 $\frac{3}{4}$	—	—	—	—	—
" 24 ...	1447	Waterbuck..	14	—	—	—	—	—
" 25 ...	1453	Hartebeeste	10 $\frac{1}{2}$	+	—	—	—	+
Oct. 6 ...	1471	Eland ...	2	+	—	+	—	—
Nov. 10 ...	1577	Warthog ...	3 $\frac{1}{2}$	—	—	—	—	—

Total 180. Infected with pathogenic trypanosomes 57 = 31·7 per cent.

In the above table an account is given of the examination of 180 wild animals shot in the fly-area adjoining the Commission's camp at Kasu. This part of the country is situated in the proclaimed Sleeping-Sickness Area of Nyasaland, which extends from the Chirua river (lat. 13° 20' S., long. 34° E.) in the north to the Lintipe river (lat. 13° 50' S., long. 34° 30' E.) in the south. It is bounded on the east by the Lake and on the west by the foot-hills. The area is about 50 miles from north to south and 25 from east to west. These figures are only approximate, as the available maps are far from correct. This is the only part of this country in which cases of the human trypanosome disease of Nyasaland, up to the present, have been found. It will be seen, then, that these animals were procured from the very heart of the Sleeping-Sickness Area.

Among the 180 animals, 57 were found to harbour pathogenic trypanosomes—31·7 per cent.

Table II gives the species of trypanosomes found in the 180 animals. Here a difficulty is encountered—the classification. The tendency in this branch of natural history, as in all others, is to multiply species.

In a previous paper* the trypanosome causing human trypanosome disease in Nyasaland was called *Trypanosoma rhodesiense*,

* Roy. Soc. Proc., 1912, B, vol. 85, p. 423. (S.S. Reports No. XV., p. 1.)

on account of the presence of posterior-nuclear forms. This trypanosome agreed in all other respects with *Trypanosoma brucei*, the common trypanosome of wild animals in South Africa, and the cause of the tsetse-fly disease, or Nagana. In order to compare the two species of trypanosomes more closely, the Commission procured, by the kindness of Dr. A. Theiler, C.M.G., Pretoria, a strain of Nagana from the same spot in Zululand where it was first discovered in 1894. Much to the surprise of the Commission it was found that *T. brucei* has quite as large a proportion of posterior-nuclear forms as *T. rhodesiense*, and that the blunt-ended character is common to both species. The Commission is therefore driven to the conclusion that *T. rhodesiense* is neither more nor less than *T. brucei*, and that the human trypanosome disease of Nyasaland is Nagana.

To this it may be objected that Nagana has never been known to attack human beings. This has probably been due to faulty diagnosis, cases in man being returned as malaria.

The pathogenic trypanosomes then, found in the blood of wild animals in Nyasaland, up to the present, by the Commission are *T. brucei* (Plimmer and Bradford) vel *rhodesiense* (Stephens and Fantham), *T. pecorum*, *T. simiae* and *T. capræ* (Kleine). *T. ingens* is also found, but this trypanosome cannot, with our present knowledge, be considered a pathogenic species to man or domestic animals.

In Table II the plus sign means that the trypanosome named at the top of the column was present in the blood. The other plus signs signify that the trypanosome was found in a thick or thin film or by inoculation of a quantity of blood from the wild animal into healthy experimental animals.

Table II.—Species of Trypanosomes found in the Blood of Wild Animals living in the Sleeping-Sickness Area, Nyasaland.

Date.	Experiment No.	Animal.	<i>T. brucei</i> vel <i>rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simiae</i> .	<i>T. capræ</i> .	<i>T. ingens</i> .	Thick film.	Thin film.	Inoculation.
1912.										
Jan. 22	41	Eland ...		+						+
" 22	41	" ...		+						+
May 19	616	Waterbuck				+			+	
" 26	591	Reedbuck...				+			+	
June 2	613	Buffalo ...		+				+		
" 23	743	Oribi ...					+	+		
" 30	783	Reedbuck...	+			+		+	+	+
July 1	779	Hartebeeste	+			+		+		+
" 5	828	Reedbuck...				+	+	+		
" 6	826	Warthog ...		+				+	+	
" 8	863	Oribi ...	+							+
" 10	860	" ...				+			+	
" 20	912	Reedbuck...		+						+
" 22	933	Warthog ...		+				+	+	
" 23	955	Hyæna ...		+						+
" 23	956	" ...		+				+		
" 23	957	Hartebeeste	+							+

Table II.—continued.

Date.	Ex- per- iment No.	Animal.	<i>T. brucei</i> vel <i>rhodesi-</i> <i>ense.</i>	<i>T. pe-</i> <i>corum.</i>	<i>T. si-</i> <i>mice.</i>	<i>T. ca-</i> <i>præ.</i>	<i>T. in-</i> <i>gens.</i>	Thick film.	Thin film.	Inocu- lation.
1912.										
July 23	958	Hartebeeste	+					+	+	
" 25	988	Reedbuck...				+		+	+	+
" 27	1000	Hartebeeste	+					+		+
" 29	1007	Duiker ...	+					+		
" 30	1013	Eland ...		+		+		+	+	+
Aug. 2	1027	Duiker ...					+	+		
" 4	1044	Eland ...		+						+
" 11	1058	Koodoo ...		+				+	+	
" 11	1061	Waterbuck		+		+		+		+
" 11	1064	Warthog ...	+					+	+	
" 18	1078	Bushbuck...		+				+	+	+
" 18	1081	" ...		+				+		
" 18	1084	" ...		+				+	+	+
" 18	1087	" ...		+		+		+	+	+
" 19	1096	Oribi ...		+						+
" 21	1139	Warthog ...			+			+	+	+
" 21	1142	Hartebeeste	+							+
" 22	1150	Reedbuck...				+		+	+	+
" 22	1153	" ...				+		+		+
" 22	1156	" ...				+		+		+
" 22	1162	" ...				+		+		+
" 24	1180	Waterbuck	+			+		+		+
" 24	1186	Warthog ...			+			+		+
" 24	1189	" ...		+				+		
" 28	1202	Eland ...		+				+	+	+
" 28	1203	Bushbuck...		+				+	+	
" 28	1210	Waterbuck	+			+		+		+
" 30	1216	Bushbuck...		+				+		
Sept. 7	1261	" ...		+						+
" 7	1264	Waterbuck	+					+	+	+
" 11	1304	Buffalo ...		+						+
" 12	1308	Warthog ...			+					+
" 13	1339	Waterbuck				+				+
" 13	1347	Reedbuck...	+					+		+
" 14	1363	" ...				+				+
" 17	1380	Koodoo ...		+						+
" 18	1388	Waterbuck				+		+	+	+
" 20	1406	"				+				+
" 20	1410	"				+			+	
" 23	1435	Reedbuck...	+							+
" 25	1453	Hartebeeste		+				+		+
Oct. 6	1471	Eland ...		+				+		+

Table III.—Species of Trypanosomes found in the Blood of Wild Animals in the Sleeping-Sickness Area, Nyasaland, and the Number of Times each was found.

Number of animals.	<i>T. brucei</i> vel <i>rhodesiense.</i>	<i>T. pecorum.</i>	<i>T. simiæ.</i>	<i>T. capræ.</i>	<i>T. ingens.</i>
180	14	26	3	20	3

In every 100 wild animals living in the Sleeping-Sickness Area, Nyasaland, taken at random, the following numbers may be expected to be found infected with these species of trypanosomes.

Table IV.—Percentage of Animals infected by the different Species of Trypanosomes.

<i>T. brucei</i> vel <i>rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simia</i> .	<i>T. capræ</i> .	<i>T. ingens</i> .
7·8	14·4	1·7	11·1	1·7

Table V.—The Species of Animals dealt with, the Total Number examined, the Number found Infected, and the Species of Trypanosomes by which they were Infected.

Animal.	Total No. examined.	No. found infected.	<i>T. brucei</i> vel <i>rhodesiense</i> .	<i>T. pe- corum</i> .	<i>T. si- miæ</i> .	<i>T. ca- præ</i> .	<i>T. in- gens</i> .
Eland ...	10	6		6		1	
Sable ...	5	0					
Waterbuck	13	9	3	1		8	
Koodoo ...	3	2		2			
Bushbuck ...	10	7		7		1	
Hartebeeste	35	6	5	1			
Reedbuck ...	19	12	3	1		9	1
Oribi ...	26	4	1	1		1	1
Duiker ...	7	2	1				1
Buffalo ...	9	2		2			
Lion ...	1	0					
Hyæna ...	3	2		2			
Elephant ...	2	0					
Warthog ...	33	7	1	3	3		
Wild Cat ...	3	0					
Porcupine...	1	0					
Total ...	180	59	14	26	3	20	3

The next table gives the percentages of the different trypanosomes occurring in the wild animals. The numbers are too small to be taken literally, but it is interesting to learn that in this fly-district the waterbuck, hartebeeste, reedbuck and duiker are dangerous neighbours to man; the eland, koodoo, bushbuck and buffalo to cattle, goats and sheep; and that the warthog is the only animal which harbours *T. simia*, the lightning destroyer of the domestic pig.

Table VI.—Percentages of Different Species of Trypanosomes harboured by Wild Animals in the Fly-area.

Animal.	No. examined.	<i>T. brucei</i> vel <i>rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simia</i> .	<i>T. capræ</i> .	<i>T. ingens</i> .
		per cent.	per cent.	per cent.	per cent.	per cent.
Eland ...	10		60		10	
Sable ...	5					
Waterbuck ...	13	23	8		61	
Koodoo ...	3		66			
Bushbuck ...	10		70		10	
Hartebeeste ...	35	14	3			
Reedbuck ...	19	16	5		47	5
Oribi ...	26	4	4		4	4
Duiker ...	7	14				14
Buffalo ...	9		22			
Lion ...	1					
Hyæna ...	3		66			
Elephant ...	2					
Warthog ...	33	3	9	9		
Wild cat ...	3					
Porcupine ...	1					

Conclusions.

1. 31·7 per cent. of the wild game in the fly-country below Kasu Hill harbour pathogenic trypanosomes.

2. The species of trypanosomes found are *T. brucei vel rhodesiense* 7·8 per cent., *T. pecorum* 14·4, *T. simia* 1·7, *T. capræ* 11·1, and *T. ingens* 1·7.

3. It is self-evident that these wild animals should not be allowed to live in "fly-country," where they constitute a standing danger to the native inhabitants and the domestic animals. It would be as reasonable to allow mad dogs to live and be protected by law in our English towns and villages. Not only should all game laws restricting their destruction in "fly-country" be removed, but active measures should be taken for their early and complete blotting out.

4. It must be strictly borne in mind that this only refers to wild animals living in fly-areas. No pathogenic trypanosomes have, up to the present, been found by the Commission in the blood of animals living in fly-free areas.

IV.—TRYPANOSOME DISEASES OF DOMESTIC ANIMALS IN NYASALAND.*

II.—*Trypanosoma capræ* (Kleine).

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; Dr. J. B. DAVEY, Nyasaland Medical Staff†; and Lady BRUCE, R.R.C.

(Received January 13, 1913.)

[PLATE 4.]

INTRODUCTION.

This species belong to the *vivax* group, which consists of three species:—*Trypanosoma uniforme*, *T. vivax*, and *T. capræ*. They are all characterised by their extreme motility; clear cell contents; large, round, terminal micronucleus; and lastly, by the fact that the *vivax* group only infects cattle, goats, and sheep, and is harmless to the smaller laboratory animals. All three develop in the proboscis of the tsetse flies and not in the alimentary tract, as do other pathogenic trypanosomes.

T. vivax is stated to be pathogenic to horses, mules, and donkeys, but there has been no opportunity of testing these animals at Kasu with *T. capræ*.

It is curious that *T. uniforme* and *T. vivax* have not been met with by the Commission in Nyasaland. This may be due to the absence of *Glossina palpalis*, which is their carrier, while *T. capræ* is carried by *G. morsitans*.

MORPHOLOGY OF *T. CAPRÆ*.

A. *Living, Unstained.*

The description given of *T. vivax* can be equally applied to this species. It is just as active in its movements and dashes across the field of the microscope with the same impetuosity.

B. *Fixed and Stained.*

The blood films were fixed, stained, and measured as previously described in the 'Proceedings.'‡

Length.—The following table gives the length of this trypanosome as found in the waterbuck, ox, goat, and sheep—500 trypanosomes in all.

* Reprinted from the Proceedings of the Royal Society, B, vol. 86.

† Dr. Davey resigned his membership of the Commission in October, 1912, before the completion of the work here recorded.

‡ 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 16 and 17. (S.S. Reports No. X., p. 12.)

Table I.—Measurements of the Length of *Trypanosoma capræ*, Nyasaland.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.			
					Ave- rage length.	Maxi- mum length.	Mini- mum length.	
1912.								
Sept. 18 ...	1388	Waterbuck	Osmic acid	Giemsa	26·8	29·0	25·0	
" 4 ...	349	Ox ...	"	"	25·9	32·0	22·0	
" 9 ...	350	" ...	"	"	25·5	30·0	18·0	
March 4 ...	175	Goat ...	"	"	23·3	28·0	20·0	
" 11 ...	263	" ...	"	"	26·1	29·0	21·0	
" 14 ...	263	" ...	"	"	27·5	30·0	24·0	
" 18 ...	200	" ...	"	"	26·2	30·0	22·0	
" 20 ...	263	" ...	"	"	26·9	30·0	22·0	
" 21 ...	247	" ...	"	"	23·7	26·0	20·0	
" 21 ...	263	" ...	"	"	24·4	29·0	21·0	
April 4 ...	339	" ...	"	"	25·1	28·0	23·0	
" 22 ...	272	" ...	"	"	23·4	27·0	21·0	
" 22 ...	339	" ...	"	"	24·6	27·0	21·0	
" 29 ...	339	" ...	"	"	27·6	31·0	25·0	
May 13 ...	339	" ...	"	"	25·5	29·0	23·0	
" 16 ...	339	" ...	"	"	24·4	27·0	23·0	
April 8 ...	348	Sheep ...	"	"	26·6	30·0	24·0	
" 11 ...	346	" ...	"	"	24·6	28·0	21·0	
" 11 ...	348	" ...	"	"	26·7	29·0	24·0	
" 25 ...	348	" ...	"	"	23·9	27·0	22·0	
May 9 ...	348	" ...	"	"	26·2	29·0	24·0	
" 13 ...	348	" ...	"	"	24·4	27·0	22·0	
" 23 ...	347	" ...	"	"	24·7	27·0	22·0	
June 13 ...	548	" ...	"	"	25·6	29·0	23·0	
July 25 ...	907	" ...	"	"	27·5	32·0	24·0	
					25·5	32·0	18·0	

The average length of *T. capræ*, Nyasaland, in different species of animals, taken from Table I., is as follows:—

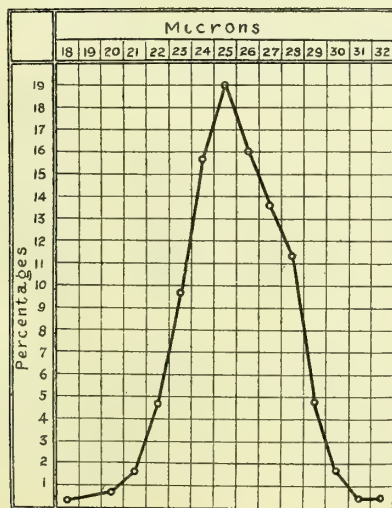
Table II.

Species of animal.				In microns.		
				Average length.	Maximum length.	Minimum length.
Waterbuck	...	...	20	26·8	29·0	25·0
Ox	...	...	40	25·7	32·0	28·0
Goat	...	...	260	25·3	31·0	20·0
Sheep	...	...	180	25·6	32·0	21·0

Table III.—Distribution in respect to Length of 500 Individuals of *T. capræ*, Nyasaland.

Animal.	In microns.																Average length.
	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.		
Waterbuck ...	—	—	—	—	—	—	—	1	9	4	4	2	—	—	—	26·8	
Ox ...	—	—	—	—	1	2	3	2	3	6	2	—	—	—	1	25·9	
” ...	1	—	—	—	1	—	4	3	4	2	4	—	1	—	—	25·5	
Goat ...	—	—	2	—	6	4	1	5	1	—	1	—	—	—	—	23·3	
” ...	—	—	—	1	—	3	1	2	2	5	4	2	—	—	—	26·1	
” ...	—	—	—	—	—	—	1	—	3	6	6	2	2	—	—	27·5	
” ...	—	—	—	—	1	1	3	4	2	1	5	2	1	—	—	26·2	
” ...	—	—	—	—	1	—	—	4	2	5	3	4	1	—	—	26·9	
” ...	—	—	1	2	—	6	4	3	4	—	—	—	—	—	—	23·7	
” ...	—	—	—	1	—	4	6	6	1	1	—	1	—	—	—	24·4	
” ...	—	—	—	—	—	4	5	4	2	2	3	—	—	—	—	25·1	
” ...	—	—	—	2	3	6	4	4	—	1	—	—	—	—	—	23·4	
” ...	—	—	—	1	—	2	7	4	5	1	—	—	—	—	—	24·6	
” ...	—	—	—	—	—	—	—	2	4	3	4	5	1	1	—	27·6	
” ...	—	—	—	—	—	2	2	7	5	2	1	1	—	—	—	25·5	
” ...	—	—	—	—	—	3	9	5	2	1	—	—	—	—	—	24·4	
Sheep ...	—	—	—	—	—	—	3	4	1	6	3	1	2	—	—	26·6	
” ...	—	—	—	1	2	2	4	4	5	1	1	—	—	—	—	24·6	
” ...	—	—	—	—	—	—	1	2	7	3	6	1	—	—	—	26·7	
” ...	—	—	—	—	4	4	5	5	1	1	—	—	—	—	—	23·9	
” ...	—	—	—	—	—	—	1	4	8	4	2	1	—	—	—	26·2	
” ...	—	—	—	—	2	1	6	9	1	1	—	—	—	—	—	24·4	
” ...	—	—	—	—	2	2	3	8	3	2	—	—	—	—	—	24·7	
” ...	—	—	—	—	—	3	5	—	4	5	2	1	—	—	—	25·6	
” ...	—	—	—	—	—	—	1	3	1	5	6	1	1	1	1	27·5	
Total ...	1	—	3	8	23	49	79	95	80	68	57	24	9	2	2		
Percentages	0·2	—	0·6	1·6	4·6	9·8	15·8	19·0	16·0	13·6	11·4	4·8	1·8	0·4	0·4		

CHART giving Curve representing the Distribution by Percentages in respect to Length of 500 Individuals of *Trypanosoma capræ*, Nyasaland.



This curve is made up of measurements from 20 specimens of trypanosomes taken from the waterbuck, 40 from the ox, 260 from the goat, and 180 from the sheep.

From this it will be seen that *T. capræ* is a monomorphic species, varying from 18 to 32 microns in length, the greatest number of individuals (19 per cent.) being 25 microns long.

Breadth.—Measured across the broadest part *T. capræ* averages 3 microns in breadth (maximum 4·25, minimum 1·75).

Shape.—*T. capræ* differs from *T. vivax* in that it is heavier built and altogether has a larger and clumsier appearance. The posterior half is swollen, and its end is bluntly angular or rounded. The anterior extremity is narrower and pointed (Plate 5).

Contents of Cell.—Clear, with a delicate alveolar structure, and free from vacuoles or granules.

Nucleus.—Oval, compact, lying about the middle of the body.

Micronucleus.—Large and round, situated, as a rule, close to the posterior extremity, but sometimes removed to a short distance.

Undulating membrane.—Much more developed than in *T. vivax*, and thrown into bolder folds and undulations.

Flagellum.—There is a well-marked free flagellum which averages 6·5 microns in length (maximum 9·5, minimum 4). No specimens have been seen without a free flagellum as stated by Kleine.

Disease set up in Cattle by T. capræ.—Only two oxen were inoculated from goats suffering from this disease. These animals showed the trypanosomes in their blood in small numbers for two months after inoculation. The trypanosomes then disappeared and have never reappeared. The two oxen at the present time are in good health and have evidently recovered. This strain of *T. capræ* cannot, therefore, be considered of much pathological importance as far as oxen are concerned, but more cases are wanted. Kleine states that cattle are immune.

Disease set up in Goats and Sheep by T. capræ.—In goats and sheep, on the other hand, *T. capræ* runs a fairly fatal course. In the list of animal experiments 36 goats and 4 sheep are given. Of the 36 goats, 15 had been infected by wild *G. morsitans* and died, on an average of, from 53 to 59 days. As the flies were fed on a goat, a monkey, and a dog, and, as a rule, three times on each animal, to ensure that all the flies fed, it is not possible to tell the exact day of infection.

Four others were inoculated with blood from infected goats or antelope, and these died, on an average, in 57 days. The remaining 17 goats are still alive after intervals of from 61 to 262 days. Of the four sheep, one died in 36 days, another lived 89 days, the third 221 days, while the fourth is still alive after 245 days.

Table IV.—Animals Susceptible to *T. capræ*.

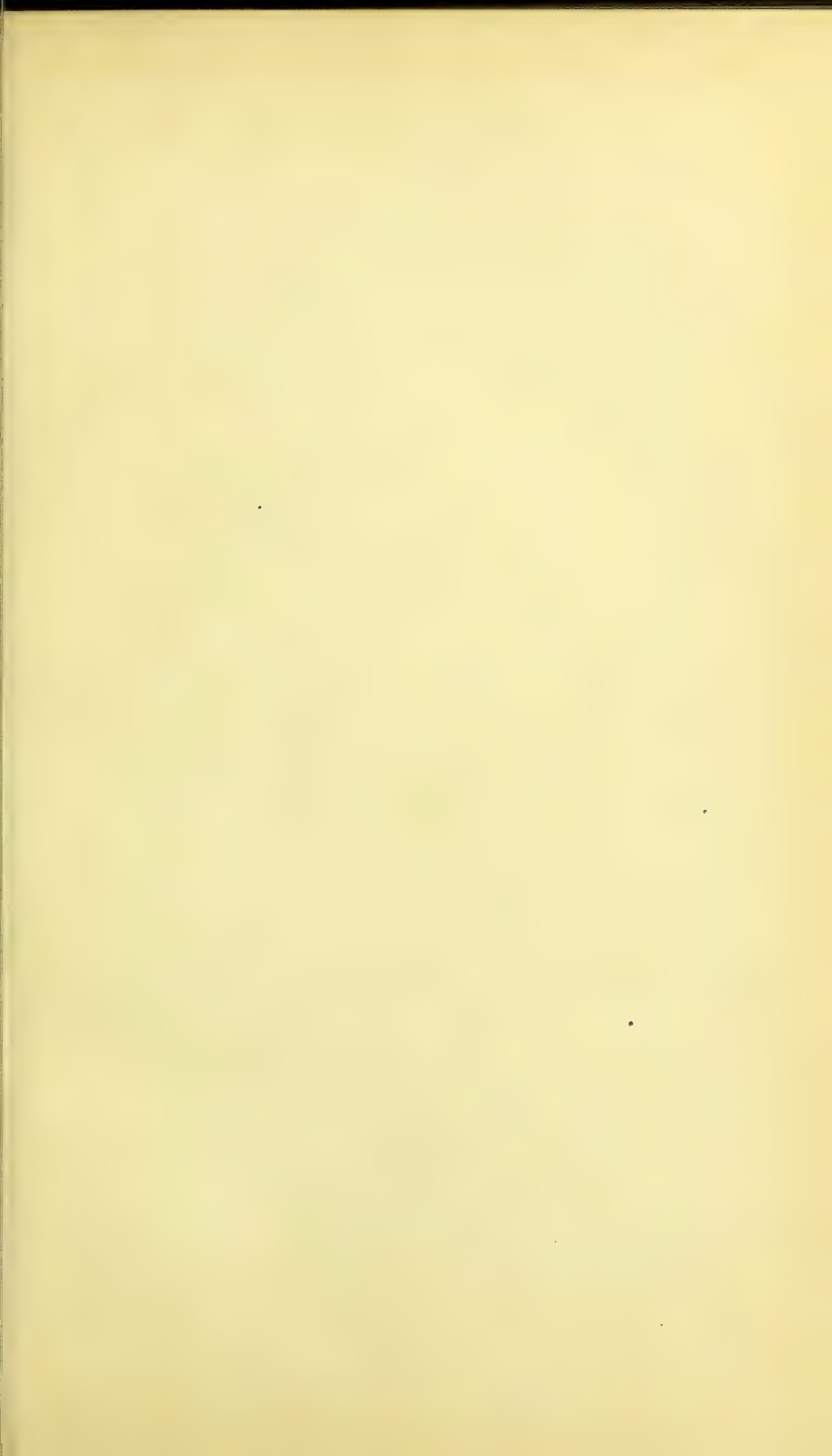
Date.	No. of experiments.	Source of virus.	Period of incubation, in days.	Duration, in days.	Remarks.
Cattle.					
1912.					
Mar. 20	349	From Goat 263 ...	20	—	Alive and well after
" 20	350	" 263 ...	10	—	245 days. These animals appear to have recovered.
Goat.					
Feb. 6	273	Wild flies ...	0-12	41-53	Died of <i>T. capræ</i> .
" 14	200	" ...	20-26	128-134	" "
" 22	281	" ...	2-17	51-66	" "
Mar. 3	272	" ...	21-29	—	Alive after 262 days.
" 11	263	Natural infection ...	?	?	Died March 22.
" 14	334	Wild flies ...	7?	—	Alive after 251 days.
" 16	335	From Goat 263 ...	5	—	" 249 "
" 16	339	" 263 ...	9	63	Died of <i>T. capræ</i> .
" 16	340	" 263 ...	16	—	Alive after 249 days.
" 20	346	" 263 ...	5	—	" 245 "
" 20	347	" 263 ...	12	—	" 245 "
" 20	348	" 263 ...	5	89	Died of <i>T. capræ</i> .
Apr. 1	398	Wild flies ...	10-21	101-112	" "
" 11	410	" ...	6-11	46-51	" "
" 12	412	" ...	0-13	0-20	" "
" 16	414	" ...	7-13	33-39	" "
" 19	422	" ...	4-10	—	Alive after 215 days.
" 24	415	" ...	13-19	46-52	Died of <i>T. capræ</i> .
" 24	420	" ...	13-19	43-49	" "
May 2	433	" ...	10	63	" "
" 4	435	" ...	13-19	55-61	" "
" 9	266	" ...	6-11	69-74	" "
" 9	269	" ...	10-18	18-26	" "
" 18	553	" ...	6-16	—	Alive after 186 days.
" 23	565	" ...	3-10	52-59	Died of <i>T. capræ</i> .
June 5	622	" ...	15	34	" "
July 25	979	From Reedbuck 988	11	—	Alive after 118 days.
Aug. 18	1039	From Bushbuck 1087	8	33	Died of <i>T. capræ</i> .
" 22	1111	From Reedbuck 1153	11	43	" "
" 22	1114	" 1156	11	—	Alive after 90 days.
" 22	1118	" 1150	11	—	" 90 "
" 22	1120	" 1162	7	—	" 90 "
Sept. 13	1342	From Waterbuck 1339	20	—	" 68 "
" 14	1366	From Reedbuck 1363	9	—	" 67 "
" 18	1391	From Waterbuck 1388	8	—	" 63 "
" 20	1409	" 1406	17	—	" 61 "
Sheep.					
July 17	907	From Goat 653 ...	5	36	Died of <i>T. capræ</i> .
Mar. 20	346	" 263 ...	5	—	Alive after 245 days.
" 20	347	" 263 ...	12	221	Died of <i>T. capræ</i> .
" 20	348	" 263 ...	5	89	" "

Table IV.—*continued.*

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration, in days.	Remarks.
Monkey.					
1912.					
Feb. 2	49	Wild flies ...	—	—	Only showed <i>T. simiae</i> .
Mar. 9	326	From Goat 175 ...	—	—	Never showed trypanosomes.
Apr. 15	405	Wild flies ...	—	—	Only showed <i>T. simiae</i> .
" 23	465	" ...	—	—	" "
" 23	467	" ...	—	—	Only " showed <i>T. pecorum</i> .
July 25	989	From Reedbuck 988	—	—	Never showed trypanosomes.
Aug. 22	1154	" 1153	—	—	" "
" 22	1157	" 1156	—	—	" "
Sept. 13	1340	From Waterbuck 1339	—	—	" "
" 14	1364	From Reedbuck 1363	—	—	" "
" 18	1389	From Waterbuck 1388	—	—	" "
" 20	1407	" 1406	—	—	" "
Dog.					
Mar. 9	319	From Goat 175 ...	—	—	Never showed trypanosomes.
" 9	320	" 175 ...	—	—	Only showed <i>T. brucei</i> .
" 9	321	" 125 ...	—	—	Only showed <i>T. pecorum</i> .
" 9	322	" 125 ...	—	—	" "
" 9	344	" 263 ...	—	—	Never showed trypanosomes.
" 9	345	" 263 ...	—	—	" "
July 25	990	From Reedbuck 988	—	—	" "
Aug. 22	1155	" 1153	—	—	" "
" 22	1158	" 1156	—	—	" "
Sept. 13	1341	From Waterbuck 1339	—	—	" "
" 14	1365	From Reedbuck 1363	—	—	" "
" 18	1390	From Waterbuck 1388	—	—	" "
Guinea-pig.					
Mar. 20	351	From Goat 263 ...	—	—	Never showed trypanosomes.
" 20	352	" 263 ...	—	—	" "
Rat.					
Mar. 20	351	From Goat 263 ...	—	—	Never showed trypanosomes.
" 20	352	" 263 ...	—	—	" "

THE CARRIER OF *T. CAPRÆ*.

The carrier of *T. capræ* in Nyasaland is *G. morsitans*. These tsetse flies in the neighbourhood of Kasu are heavily infected with this trypanosome.





Goat.

Sheep.

Ox.

Waterbuck.

Trypanosoma capræ (Kleine).

In the experiments made to ascertain with what trypanosomes the wild flies are naturally infected, *T. capræ* was found in 61 per cent.

The development of this trypanosome in *G. morsitans* will be dealt with in a future paper; suffice it to say here that it is restricted to the proboscis and runs a course of from 16 to 20 days.

THE HOST OR RESERVOIR OF *T. CAPRÆ*.

Up to the present 180 specimens of wild game living in the Nyasaland Sleeping-Sickness Area have been examined. Of these 19, or 10·5 per cent., harboured *T. capræ*. The animals were reedbuck, waterbuck, eland, and bushbuck.

Conclusions.

1. *T. capræ* belongs to the same group as *T. vivax* and *T. uniforme*, and affects the same animals—cattle, goats, and sheep. Monkeys, dogs, and the smaller laboratory animals are immune.

2. The carrier is *G. morsitans*.

3. The reservoir of the virus is the wild game living in the "fly-country."

DESCRIPTION OF PLATE 4.

Trypanosome capræ (Kleine).—Large, heavily built body; posterior extremity swollen, bluntly angular, or rounded; anterior extremity pointed; nucleus oval, compact; micronucleus large, round, situated, as a rule, close to posterior extremity; undulating membrane marked, thrown into bold folds; flagellum well marked, free, average 6·5 microns in length. × 2000.

V.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRY- PANOSOME CAUSING DISEASE IN MAN IN NYASA- LAND. I.—THE HUMAN STRAIN.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; and Lady BRUCE, R.R.C.

(Received February 8, 1913.)

INTRODUCTION.

In order to gain a general idea of this important species of trypanosome, it will be necessary to study as many individual strains as possible. It may be thought unnecessary to describe each strain so much in detail, but without this it will be impossible to get any order out of the chaos which rules at present

* Reprinted from the Proceedings of the Royal Society, B, vol. 86.

in the classification of the African species of trypanosomes pathogenic to man and the domestic animals.

Up to the present the Commission have only had an opportunity of working with five human strains. Four of these are from natives infected in the Sleeping-Sickness Area, Nyasaland, the fifth from an European who contracted the disease in Portuguese East Africa. It is intended, in later papers, to describe five strains from wild game and the same number from the tsetse fly, *Glossina morsitans*.

The human strains are named: I, Mkanyanga; II, E—; III, Chituluka; IV, Chipochola; and V, Chibibi.

I. Morphology of Strain I, Mkanyanga.

This has already been dealt with in a previous paper.*

I. Morphology of Strain II, E—.

The following table gives the average length of this trypanosome as found in goats, sheep, monkeys, dogs and rats, 1,500 trypanosomes in all, and also the length of the longest and shortest:—

Table I.—Measurements of the Length of the Trypanosome of Strain II, E—.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·2	36·0	15·0

The average length of the trypanosome of Strain II, in different species of animals, is as follows:—

Table II.

Species of animal.			Number of trypanosomes measured.	In microns.		
				Average length.	Maximum length.	Minimum length.
Goat	...	...	60	20·7	34·0	15·0
Sheep	...	...	20	21·3	28·0	18·0
Monkey	...	...	160	22·9	36·0	17·0
Dog	...	...	260	21·8	31·0	17·0
Rat	...	...	1000	23·1	32·0	17·0

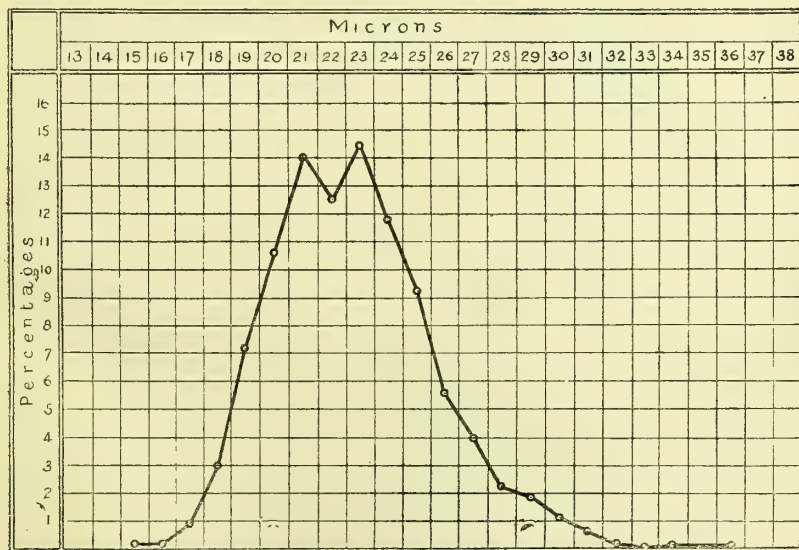
* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 423. (S.S. Reports No. XV., p. 1.)

Table III.—Distribution in respect to Length of 1,500 Individuals of the Trypanosome of Strain II, E—.

	In microns.										
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Total ...	2	2	12	55	108	159	210	188	215	177	138
Percentage...	0.2	0.2	0.9	3.0	7.2	10.6	14.0	12.6	14.4	11.8	9.2

	In microns.										
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.
Total ...	83	60	34	26	18	8	3	—	1	—	1
Percentage...	5.6	4.0	2.3	1.8	1.2	0.6	0.2	—	0.1	—	0.1

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,500 Individuals of the Trypanosome of Strain II, E—.



This curve is made up of measurements from 60 specimens of trypanosomes taken from the goat, 20 from the sheep, 160 from the monkey, 260 from the dog, and 1,000 from the rat.

In a previous paper it was suggested that 1,000 trypanosomes taken at random would be a suitable number to plot a curve from, for purposes of comparison. This is done in Chart 2.

The taking away of 500 rat trypanosomes has changed, to a great extent, the character of the curve. There is no resemblance between this curve and that given on Chart 1 of Strain I, Mkanyanga. If the two strains, I and II, belong to the same species, then little help can be expected from this system of measurement in classifying trypanosomes.

It has been suggested by Dr. J. W. W. Stephens that the measurements should be made from one animal, and he proposed the tame rat as a suitable species. There seems much to be said in favour of this. Practically, his proposal is that a series of slides should be made with blood taken on 10 consecutive days from a single rat, and that 100 trypanosomes should be drawn each day. But it is no light task to draw 1,000 trypanosomes at a magnification of 2,000, and afterwards to measure them. We have therefore made a compromise and measure 60 trypanosomes on nine consecutive days, beginning from the day the parasites first appear in the blood. In order to deal with a round number (500) only 20 are measured on the ninth day.

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,000 Individuals of the Trypanosome of Strain II, E—.

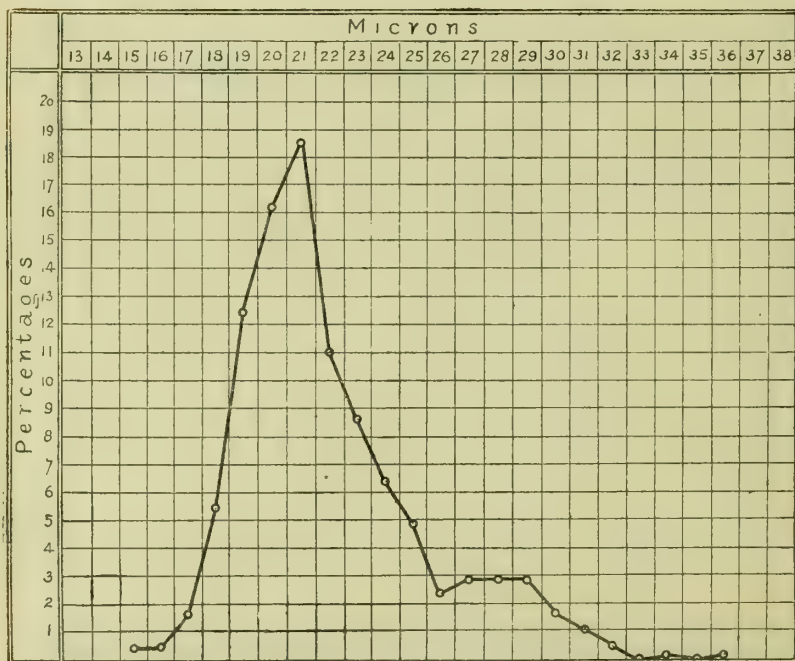
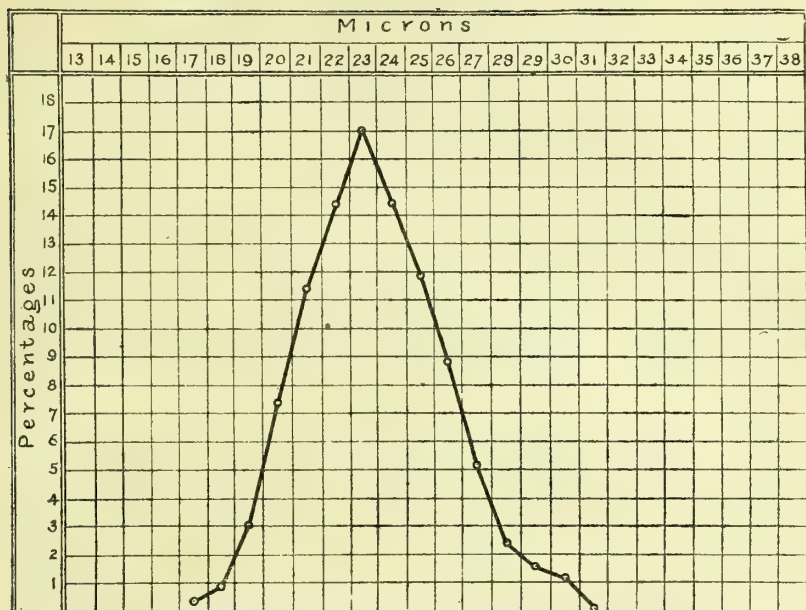


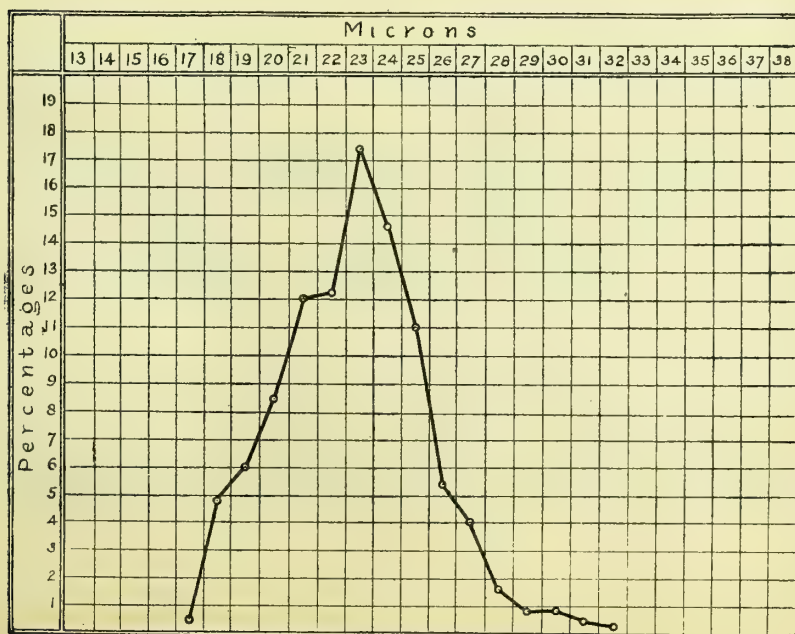
CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, E—, taken on nine consecutive days from Rat 728.



This makes a symmetrical curve, which ascends and descends by fairly regular steps, but with little likeness to Charts 1 and 2.

In an organism low in the scale of nature, such as this, subject to great variation in form, it might be thought that it would not be likely to behave in any two rats in the same way. The following chart shows that this is not so, but that, on the contrary, the same strain of trypanosome planted in two different animals of the same species grows in a remarkably similar way.

CHART 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, E—, taken on nine consecutive days from Rat 726.



It is remarkable how much alike these last two curves are. If curves made in this way from different strains of one species of trypanosome showed the same degree of similarity, this method would certainly be useful for purposes of classification. But, as we have seen, the curve of Strain II has no resemblance to that of Strain I, and it will be found that each human strain of this species of trypanosome differs, more or less, when subjected to this method of measurement.

As the occurrence of posterior-nuclear forms has been made the distinguishing character between *Trypanosoma brucei*, *gambiense*, and *rhodesiense*, it will be of interest to note the percentage of these forms in the various strains. The method used is to count the number of posterior nuclears in 1,000 short and stumpy forms in 10 specimens of a single rat's blood taken, as near as possible, on 10 consecutive days.

Table IV.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain II, E—.

Date.				Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.						
June	25	...	...	728	Rat	10
"	26	...	...	728	"	17
"	27	...	...	728	"	3
"	29	...	...	728	"	9
July	1	...	...	728	"	5
"	2	...	...	728	"	5
"	3	...	...	728	"	9
"	4	...	...	728	"	3
"	5	...	...	728	"	18
"	6	...	...	728	"	14
Average				...	...	9.3

In regard to breadth, shape, contents of cell, nucleus, micro-nucleus, undulating membrane and flagellum, it is not proposed to describe these characters separately for each strain, as was done in Strain I. Suffice it to say that no difference can be made out in regard to these particulars on comparing the five strains. The same posterior-nuclear and blunt-ended forms are present in all.

III. Morphology of Strain III, Chituluka.

The following table gives the average length of this trypanosome as found in the goat, monkey, dog and rat, 1,500 trypanosomes in all, and also the length of the longest and shortest:—

Table V.—Measurements of the Length of the Trypanosome of Strain III, Chituluka.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	26·1	38·0	15·0

The average length of the trypanosome of Strain III, in different species of animals, is as follows:—

Table VI.

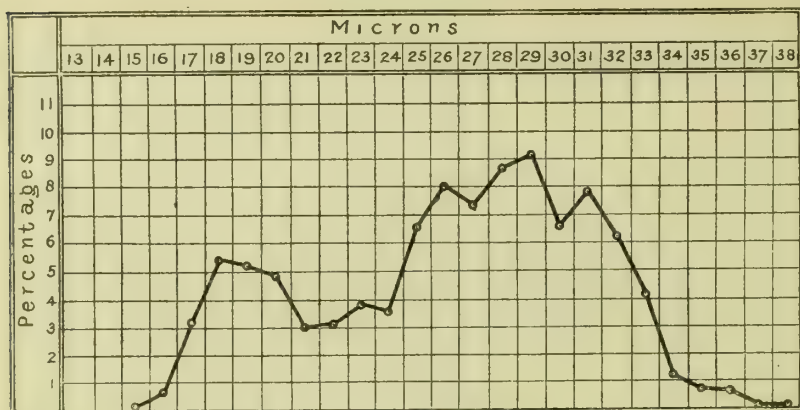
Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	26·9	32·0	16·0
Monkey	160	27·7	36·0	16·0
Dog	260	24·1	35·0	16·0
Rat	1000	26·4	38·0	15·0

Table VII.—Distribution in respect to Length of 1,500 Individuals of the Trypanosome of Strain III, Chituluka.

	In microns.											
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.
Total	1	8	48	81	78	71	44	46	56	53	98	120
Percentages ...	0·1	0·6	3·2	5·4	5·2	4·8	3·0	3·1	3·8	3·6	6·6	8·0

	In microns.											
	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.
Total	111	128	138	99	117	91	63	27	11	9	1	1
Percentages ...	7·4	8·6	9·2	6·6	7·8	6·2	4·2	1·1	0·7	0·6	0·1	0·1

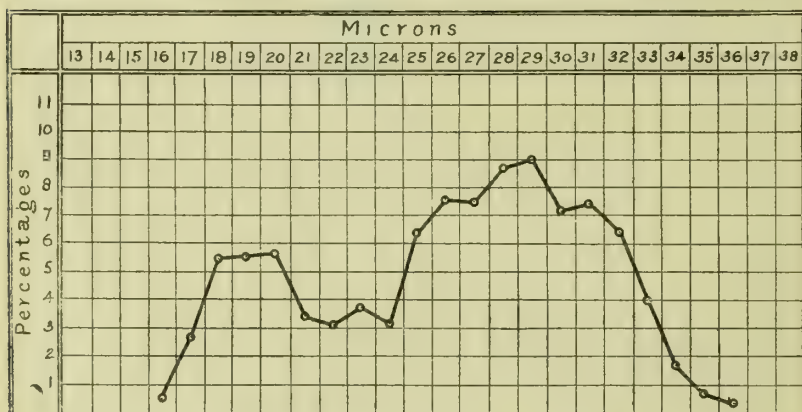
CHART 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,500 Individuals of the Trypanosome of Strain III, Chituluka.



This curve is made up of measurements from 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 1,000 from the rat. It resembles that of Strain I, and differs absolutely from Strain II.

As in the case of Strain II, E—, a curve is also given of 1,000 individuals of this strain.

CHART 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,000 Individuals of the Trypanosome of Strain III, Chituluka.



This curve, made up of 1,000 individuals, is very similar to the previous one of 1,500. It is made up of 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog and 500 from the rat.

The two following curves represent measurements of 500 trypanosomes taken from each of two rats.

CHART 7.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Chituluka, taken on nine consecutive days from Rat 952.

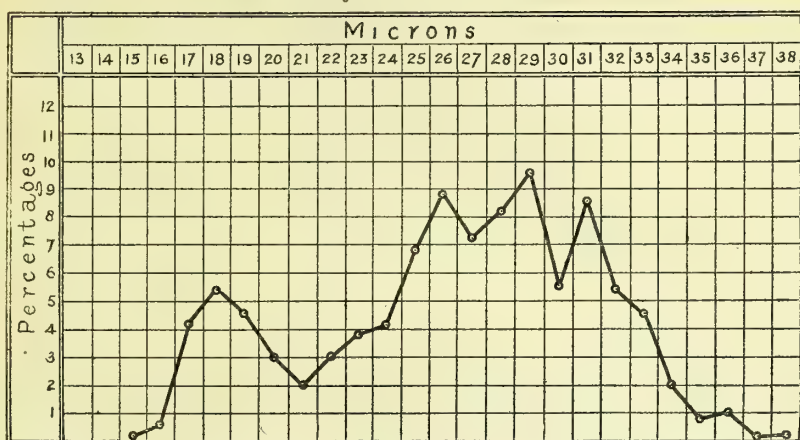
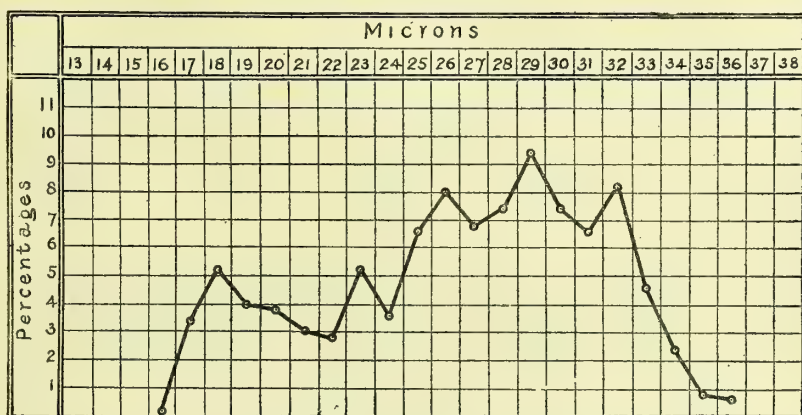


CHART 8.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Chituluka, taken on nine consecutive days from Rat 953.



These last two curves from different rats also closely resemble each other. It is curious and striking that the same strain of trypanosome growing in two different animals should show this remarkable similarity.

Table VIII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain III, Chituluka.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
August 2 ...	953	Rat	4
" 3 ...	953	"	6
" 6 ...	953	"	3
" 7 ...	953	"	8
" 8 ...	953	"	6
" 9 ...	953	"	13
" 10 ...	953	"	32
Average ...			10.3

IV. Morphology of Strain IV, Chipochola.

The following table gives the average length of this trypanosome as found in goats, monkeys, dogs and rats, 1,000 trypanosomes in all, and also the length of the longest and shortest:—

Table IX.—Measurements of the Length of the Trypanosome of Strain IV, Chipochola.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·5	34·0	15·0

The average length of the trypanosome of Strain IV, in different species of animals, is as follows:—

Table X.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	20·4	29·0	15·0
Monkey	160	22·0	34·0	16·0
Dog	260	20·9	31·0	15·0
Rat	500	22·5	34·0	15·0

Table XI.—Distribution in respect to Length of 1,000 Individuals of the Trypanosome of Strain IV, Chipochola.

			In microns.									
			15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	...	...	2	4	32	68	110	101	109	106	95	95
Percentages		...	0·2	0·4	3·2	6·8	11·0	10·1	10·9	10·6	9·5	9·5

			In microns.									
			25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	...	...	74	64	50	38	26	16	5	3	1	1
Percentages		...	7·4	6·4	5·0	3·8	2·6	1·6	0·5	0·3	0·1	0·1

Table XII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IV, Chipochola.

Date.				Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.						
September	20	...	...	1337	Rat	1
"	23	...	...	1337	"	1
"	24	...	...	1337	"	2
"	25	...	...	1337	"	4
"	26	...	...	1337	"	0
"	28	...	...	1337	"	0
"	29	...	...	1337	"	1
"	30	...	...	1337	"	14
October	1	...	...	1337	"	5
"	2	...	...	1337	"	5
Average				...	...	3.3

V. Morphology of Strain V, Chibibi.

The following table gives the average length of this trypanosome as found in goats, monkeys, dogs and rats, 1,000 in all, and also the length of the longest and shortest:—

Table XIII.—Measurements of the Length of the Trypanosome of Strain V, Chibibi.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22.4	37.0	15.0

The average length of the trypanosome of Strain V, in different species of animals, is as follows:—

Table XIV.

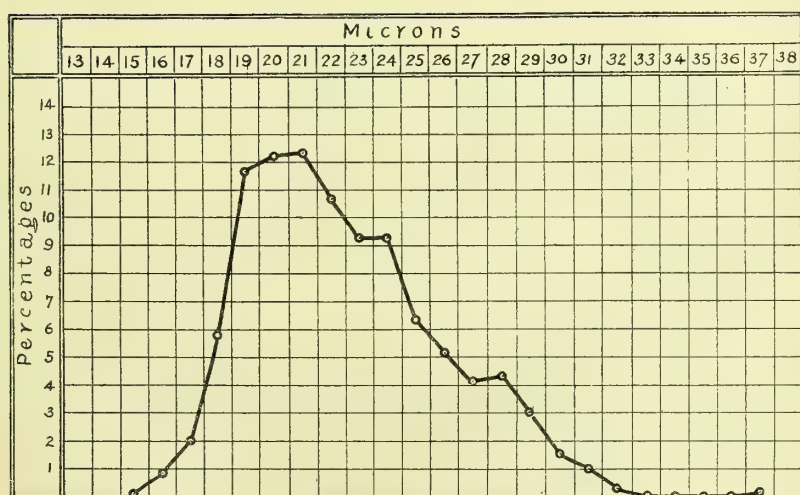
Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	19.9	31.0	16.0
Monkey	160	21.8	32.0	15.0
Dog	260	20.6	37.0	16.0
Rat	500	24.0	32.0	18.0

Table XV.—Distribution in respect to Length of 1,000 Individuals of the Trypanosome of Strain V, Chibibi.

	In microns.											
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	
Total ...	1	8	20	58	117	122	123	107	93	93	63	
Percentages ...	0.1	0.8	2.0	5.8	11.7	12.2	12.3	10.7	9.3	9.3	6.3	

	In microns.											
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.
Total ...	51	41	43	30	16	10	3	—	—	—	—	1
Percentages ...	5.1	4.1	4.3	3.0	1.6	1.0	0.3	—	—	—	—	0.1

CHART 11.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,000 Individuals of the Trypanosome of Strain V, Chibibi.



This curve is made up of 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 500 from the rat.

CHART 12.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Chibibi, taken on nine consecutive days from Rat 1660.

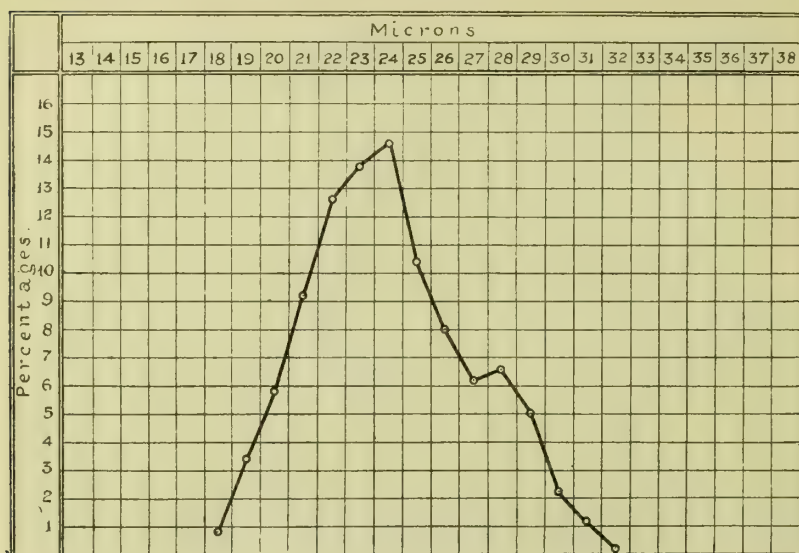


Table XVI.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain V, Chibibi.

Date.				Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.						
December	3	...	...	1660	Rat	0
"	4	...	...	1660	"	34
"	5	...	...	1660	"	2
"	8	...	...	1660	"	6
"	9	...	...	1660	"	8
"	10	...	...	1660	"	23
"	11	...	...	1660	"	31
"	12	...	...	1660	"	28
"	13	...	...	1660	"	27
"	14	...	...	1660	"	32
Average						21.1

Comparison of the Human Strains.

The following table gives the average length of this trypanosome in the five human strains under consideration, as found in goats, sheep, monkeys, dogs and rats, 6,200 trypanosomes in all, and also the length of the longest and shortest:—

Table XVII.—Measurements of the Length of the Trypanosomes of the five Human Strains. The trypanosomes have been taken from various animals.

Date.	Strain.	Name.	Number of trypanosomes.	Animals.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	I	Mkanyanga	1220	Various	24.1	36.0	14.0
1912	II	E—	1500	"	22.2	36.0	15.0
1912	III	Chituluka	1500	"	26.1	38.0	15.0
1912	IV	Chipochola	1000	"	22.5	34.0	15.0
1912	V	Chibibi	1000	"	22.4	37.0	15.0
			6220		23.5	38.0	14.0

It must be acknowledged that, in spite of the fairly large number of trypanosomes measured, there is a marked difference in the average length of the five human strains—from 22.2 to 26.1 microns. Strains II, IV and V are similar, varying only from 22.2 to 22.5.

This difference in average length may be due to slight variations having taken place in the different strains, resulting from the passage through more or less resistant man. There is no evidence that this variation is due to treatment by atoxyl or other drugs. It has been shown that the same strain grown in two animals of the same species gives like results.

Table XVIII.—Measurements of the Length of the Trypanosomes of the five Human Strains. The trypanosomes have been taken from the rat alone.

Date.	Strain.	Name.	Number of trypanosomes.	Animal.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	I	Mkanyanga	600	Rat	25.1	35.0	16.0
1912	II	E—	1000	"	23.1	32.0	17.0
1912	III	Chituluka	1000	"	26.4	38.0	15.0
1912	IV	Chipochola	500	"	22.5	34.0	15.0
1912	V	Chibibi	500	"	24.0	32.0	18.0
			3600		24.2	38.0	15.0

Comparison of the Curves from the Five Human Strains.

It must also be confessed that, on comparing the five curves one with another, they do not give as much assistance in classifying this species of trypanosome as was hoped. Curves I and

III are alike, and coincide with that prepared by Dr. Stephens from the case of Armstrong in Liverpool, whereas Curves II, IV and V approach more to the type described by Kinghorn and Yorke from the Luangwa Valley.

Table XIX.—Distribution in respect to Length of 6,220 Individuals of the five Human Strains. The trypanosomes have been taken at random from various animals.

	In microns.												
	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	
Strains I-V ...	1	10	41	154	325	494	528	577	512	525	511	464	
Percentages ...	—	0.2	0.7	2.5	5.3	8.0	8.4	9.3	8.3	8.4	8.3	7.5	

	In microns.													
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.	
Strains I-V ...	425	372	347	307	198	167	123	77	36	12	11	2	1	
Percentages ...	6.8	6.0	5.6	4.9	3.1	2.7	2.0	1.0	0.6	0.2	0.2	—	—	

CHART 13.—Curve representing the Distribution, by Percentages, in respect to Length, of 6,220 Individuals of the Human Strain of the Trypanosome causing Disease in Man in Nyasaland. The trypanosomes have been taken from various animals.

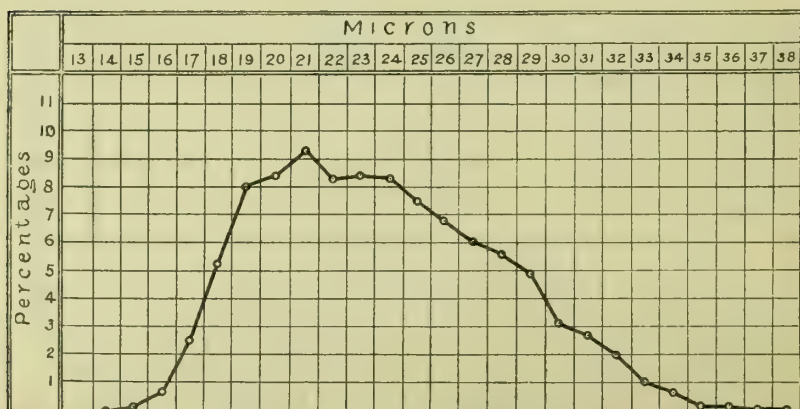
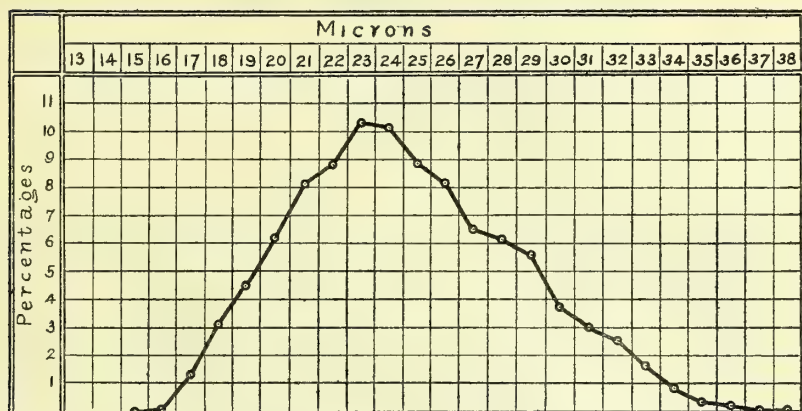


CHART 14.—Curve representing the Distribution, by Percentages, in respect to Length, of 3,600 Individuals of the Human Strain of the Trypanosome causing Disease in Man in Nyasaland, taken from the rat alone.



Curves 13 and 14 will be found of use when the human strain of this species of trypanosome is compared with the Wild Game and the Wild *Glossina morsitans* strains.

Table XX.—Comparison of the Percentages of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human Strain.

Experiment No.	Strain.	Name.	Animal.	Percentage among short and stumpy forms.
—	I	Mkanyanga	Rat	34.1
728	II	E—	"	9.3
953	III	Chituluka	"	10.3
1337	IV	Chipochola	"	3.3
1660	V	Chibibi	"	32.0
Average				17.8

It is to be noted that in the human strain the percentage of posterior-nuclear forms varies greatly, although the method of enumeration is the same in each case. This presence of posterior-nuclear forms would have been accepted a few months ago as sufficient proof that the species dealt with was *T. rhodesiense*. Since then posterior-nuclear forms have been reported as occurring in *T. brucei* from Egypt, Uganda and Zululand. In a strain lately obtained by Theiler from the same spot in Zululand where this species was originally discovered in 1894, this percentage rose to the highest yet recorded.

Conclusions.

1. The five human strains of this trypanosome, isolated from four natives in Nyasaland and one European in Portuguese East Africa, belong to the same species.

2. This species is *T. rhodesiense* (Stephens and Fantham).
3. Evidence is accumulating that *T. rhodesiense* and *T. brucei* (Plimmer and Bradford) are identical.

VI.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND.—THE WILD-GAME STRAIN.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; and Lady BRUCE, R.R.C.

(Received February 24, 1913.)

INTRODUCTION.

Trypanosomes of this species, isolated from five antelope, are here described and compared with the Human strains which formed the subject of a previous paper.† The Wild-game strains were isolated by injecting blood of antelope into susceptible animals. The blood was, as a rule, injected into a healthy goat, monkey, and dog, and from these other animals were inoculated.

Trypanosomes from the following species were studied: reedbuck, waterbuck, oribi, and hartebeeste. In these experiments, with the exception of the oribi, the three inoculated animals became infected. In the case of the oribi the blood was inoculated into a monkey and dog, no goat being available, and the monkey alone took the disease.

I. Morphology of Strain I., Reedbuck.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table I.—Measurements of the Length of the Trypanosome of Strain I., Reedbuck.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	21·7	34·0	16·0

* Reprinted from the Proceedings of the Royal Society, B, Vol. 86.

† Proceedings of the Royal Society, B, Vol. 86. (S.S. Reports No. XV., p. 31.)

Table II.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain I, Reedbuck.

	In microns.								
	16	17.	18.	19.	20.	21.	22	23.	24.
Total ...	5	30	57	90	81	53	27	17	16
Percentages...	1·0	6·0	11·4	18·0	16·2	10·6	5·4	3·4	3·2

	In microns.									
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total ...	18	23	18	25	18	9	8	2	2	1
Percentages...	3·6	4·6	3·6	5·0	3·6	1·8	1·6	0·4	0·4	0·2

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain I, Reedbuck, taken on nine consecutive days from Rat 847.

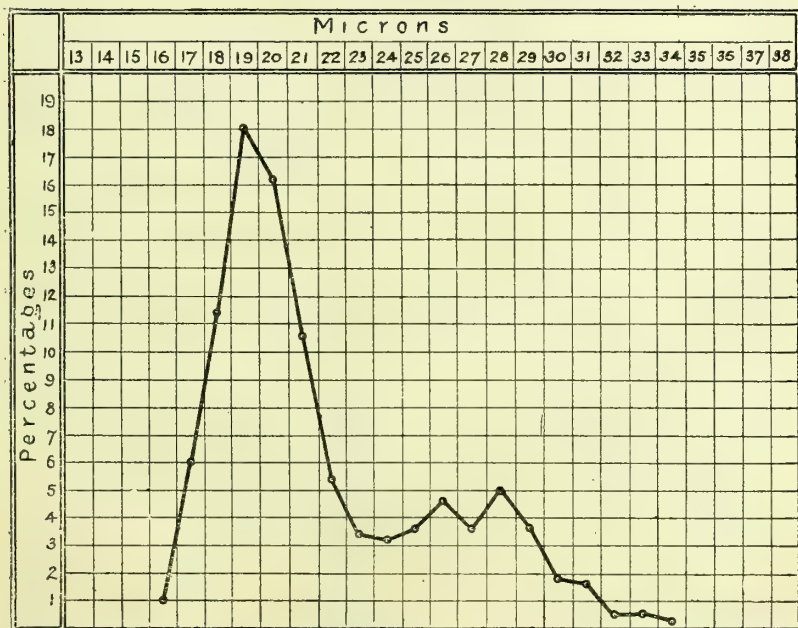


Table III.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain I, Reedbuck.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
July 17	847	Rat	4
" 20	847	"	2
" 22	847	"	2
" 23	847	"	4
" 24	847	"	9
" 25	847	"	10
" 26	847	"	19
" 27	847	"	22
" 29	847	"	4
Average			8.4

II. Morphology of Strain II, Waterbuck.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table IV.—Measurements of the Length of the Trypanosome of Strain II, Waterbuck.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	23.5	33.0	16.0

Table V.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain II, Waterbuck.

	In microns.								
	16.	17	18.	19.	20.	21.	22.	23.	24.
Total ...	1	2	8	26	59	74	58	58	44
Percentages...	0.2	0.4	1.6	5.2	11.8	14.8	11.6	11.6	8.8

Table V.—*continued.*

		In microns.								
		25.	26.	27.	28.	29.	30.	31.	32.	33.
Total	...	27	34	33	26	17	19	9	3	2
Percentages...		5.4	6.8	6.6	5.2	3.4	3.8	1.8	0.6	0.4

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, Waterbuck, taken on nine consecutive days from Rat 1220.

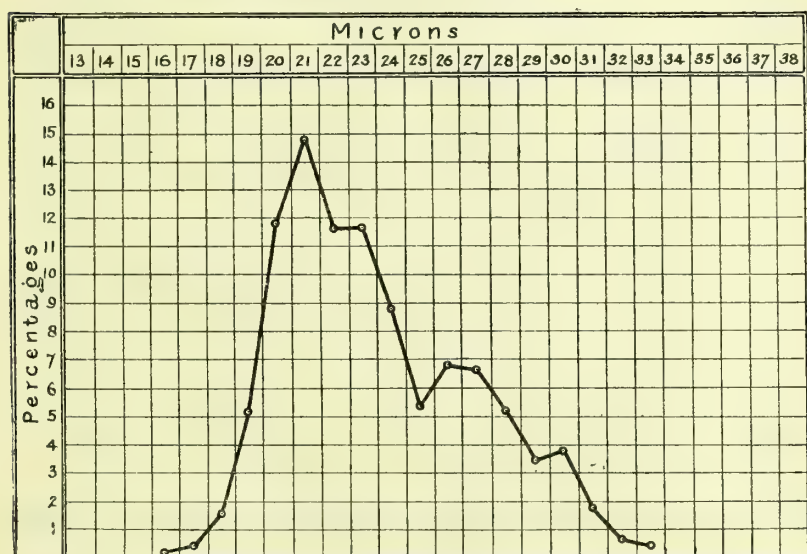


Table VI.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain II, Waterbuck.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
September 7	1220	Rat	7
" 8	1220	"	2
" 12	1220	"	12
" 16	1220	"	48
" 17	1220	"	39
" 18	1220	"	45
" 19	1220	"	21
" 20	1220	"	50
" 23	1220	"	36
" 24	1220	"	47
Average			30.7

III. *Morphology of Strain III, Oribi.*

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table VII.—Measurements of the Length of the Trypanosome of Strain III, Oribi.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	21·6	33·0	16·0

Table VIII.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain III, Oribi.

	In microns.								
	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total ...	1	10	22	77	109	90	57	28	19
Percentages...	0·2	2·0	4·4	15·4	21·8	18·0	11·4	5·6	3·8

	In microns.								
	25.	26.	27.	28.	29.	30.	31.	32.	33.
Total ...	23	15	21	14	6	5	2	—	1
Percentages...	4·6	3·0	4·2	2·8	1·2	1·0	0·4	—	0·2

CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Oribi, taken on nine consecutive days from Rat 992.

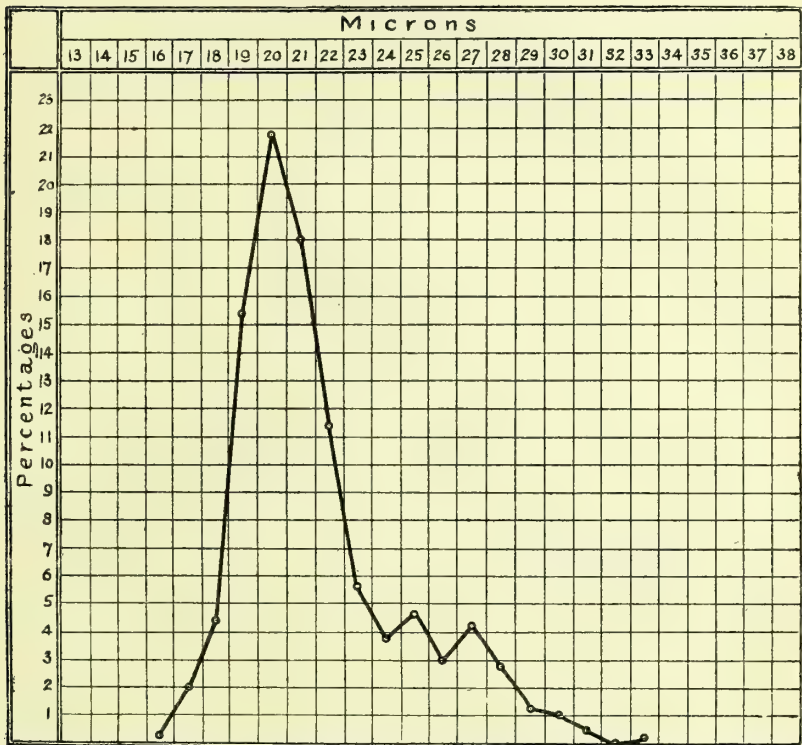


Table IX.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain III, Oribi.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
August 1	992	Rat	12
" 2	992	"	14
" 3	992	"	10
" 5	992	"	34
" 6	992	"	15
" 7	992	"	42
" 9	992	"	29
" 10	992	"	52
" 12	992	"	42
" 13	992	"	53
Average			30.3

IV. Morphology of Strain IV, Hartebeeste.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table X.—Measurements of the Length of the Trypanosome of Strain IV, Hartebeeste.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	23·5	35·0	18·0

Table XI.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain IV, Hartebeeste.

	In microns.									
	18.	19.	20.	21.	22.	23.	24.	25.	26.	
Total ...	1	12	53	80	92	53	46	45	28	
Percentages...	0·2	2·4	10·6	16·0	18·4	10·6	9·2	9·0	5·6	

	In microns.									
	27.	28.	29.	30.	31.	32.	33.	34.	35.	
Total ...	25	21	10	19	6	6	2	—	1	
Percentages...	5·0	4·2	2·0	3·8	1·2	1·2	0·4	—	0·2	

CHART 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain IV, Hartebeeste, taken on nine consecutive days from Rat 849.

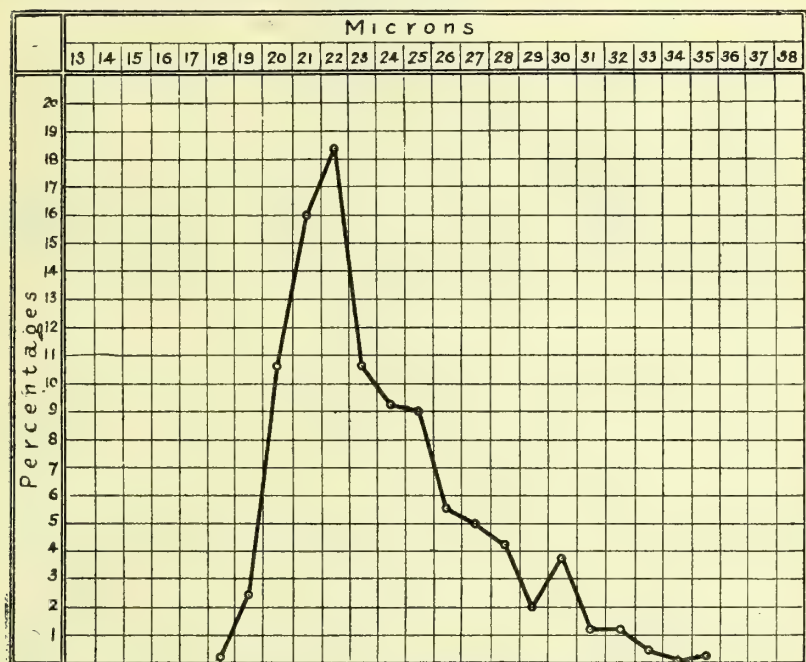


Table XII.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IV, Hartebeeste.

Date.				Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.						
July	19	...	...	849	Rat	15
"	20	...	...	849	"	18
"	22	...	...	849	"	8
"	23	...	...	849	"	16
"	24	...	...	849	"	26
"	26	...	...	849	"	49
"	29	...	...	849	"	16
"	30	...	...	849	"	38
"	31	...	...	849	"	52
August	1	...	...	849	"	45
Average						28.3

V. Morphology of Strain V, Hartebeeste.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table XIII.—Measurements of the Length of the Trypanosome of Strain V, Hartebeeste.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·6	34·0	15·0

Table XIV.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain V, Hartebeeste.

		In microns.									
		15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	...	1	1	11	30	47	79	51	51	44	37
Percentages...		0·2	0·2	2·2	6·0	9·4	15·8	10·2	10·2	8·8	7·4

		In microns.									
		25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	...	36	35	28	24	11	3	8	1	—	2
Percentages...		7·2	7·0	5·6	4·8	2·2	0·6	1·6	0·2	—	0·4

CHART 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Hartebeeste, taken on nine consecutive days from Rat 1022.

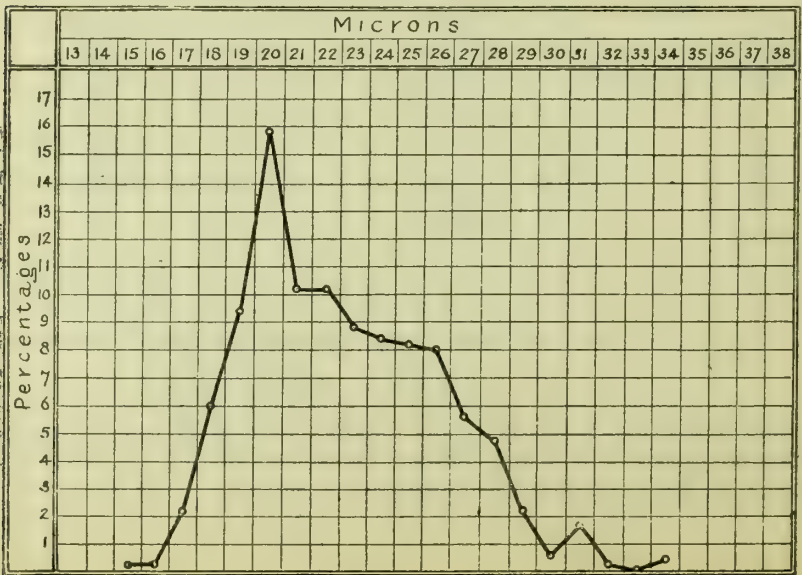


Table XV.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain V, Hartebeeste.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
August 21	1022	Rat	18
" 22	1022	"	7
" 23	1022	"	11
" 24	1022	"	30
" 26	1022	"	42
" 27	1022	"	55
" 28	1022	"	34
" 29	1022	"	54
September 2	1022	"	50
Average			33·4

Comparison of the Wild-game Strains with one another.

Table XVI.—Measurements of the Length of the Trypanosome of the Wild-game Strains.

Date.	Experiment No.	Animal.	No. measured.	From what animal.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	783	Reedbuck.	500	Rat	21·7	34·0	16·0
1912	1180	Waterbuck	500	"	23·5	33·0	16·0
1912	863	Oribi ...	500	"	21·6	33·0	16·0
1912	799	Harte-beeste.	500	"	23·5	35·0	18·0
1912	957	"	500	"	22·6	34·0	15·0
			2,500	Average	22·6	35·0	15·0

Comparison of the Curves of the Wild-game Strains.

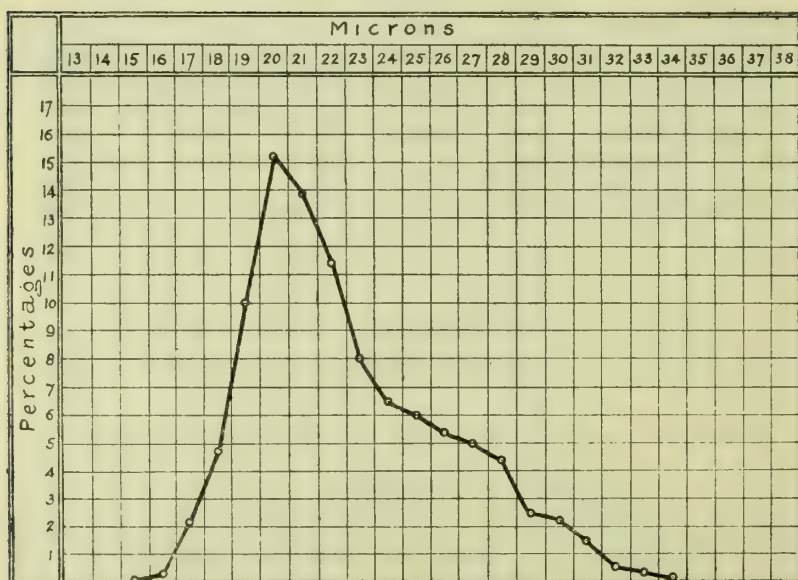
Unlike the curves of the Human strains, these are all remarkably alike, and there can be little doubt that the same species of trypanosome is being dealt with in all five of the Wild-game strains. The Wild-game curves resemble Strains II, IV, and V of the Human strains, described in a former paper, and also those found by Kinghorn and Yorke in the Luangwa Valley.

Table XVII.—Distribution in respect to Length of 2,500 Individuals of the Trypanosome of the five Wild-game Strains.

		In microns.									
		15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	...	1	8	53	118	252	381	348	285	200	162
Percentages..	—	0.3	2.1	4.7	10.0	15.2	13.9	11.4	8.0	6.5	

		In microns.											
		25.	26.	27.	28	29.	30.	31.	32.	33.	34.	35.	
Total	...	149	135	125	110	62	55	53	12	7	3	1	
Percentages..	6.0	5.4	5.0	4.4	2.5	2.2	1.5	0.5	0.3	0.1	—		

CHART 6.—Composite Curve representing the Distribution, by Percentages, in respect to Length, of 2,500 Individuals of the Trypanosome of the Wild-game Strains.



This composite curve resembles the Human Strain II, E—

Table XVIII.—Comparison of the Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosomes of the five Wild-game Strains.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912	783	Reedbuck	8·4
1912	1180	Waterbuck... ..	30·7
1912	863	Oribi	30·3
1912	779	Hartebeeste	28·3
1912	957	"	33·4
Average			26·2

It is evident from these tables and charts that the various strains of this trypanosome, as they occur in wild game, are remarkably alike. This is what might be expected. Here the trypanosome is at home: it is leading a natural life. It may be supposed to be saved from variation by constantly passing and repassing between the antelope and the tsetse fly.

Comparison of the Human Strain with the Wild-game Strain.

Table XIX.—Average Length of the Trypanosome of the Human and Wild-game Strains.

Strain.	Number of trypanosomes measured.	Animal.	In microns.		
			Average length.	Maximum length.	Minimum length
Human	3,600	Rat	24·2	38·0	15·0
Wild-game	2,500	"	22·6	35·0	15·0

The length of the trypanosomes of the Human strain found in white rats only is included in this table, in order to permit of comparison with the Wild-game strain, which is also taken from rats.

The curves (Chart 7) differ from each other in such a marked manner as to be of no use in deciding as to the identity of the Human and Wild-game strains. In spite of this, however, by a comparison of the two strains morphologically and by the susceptibility of the different experimental animals to their pathogenic action, the Commission are driven for the present to the decision that the two strains belong to the same species of trypanosome.

CHART 7.—Curves representing the Distribution, by Percentages, in respect to Length, of 3,600 Individuals of the Trypanosome of the Human Strain, and 2,500 of the Wild-game Strain.

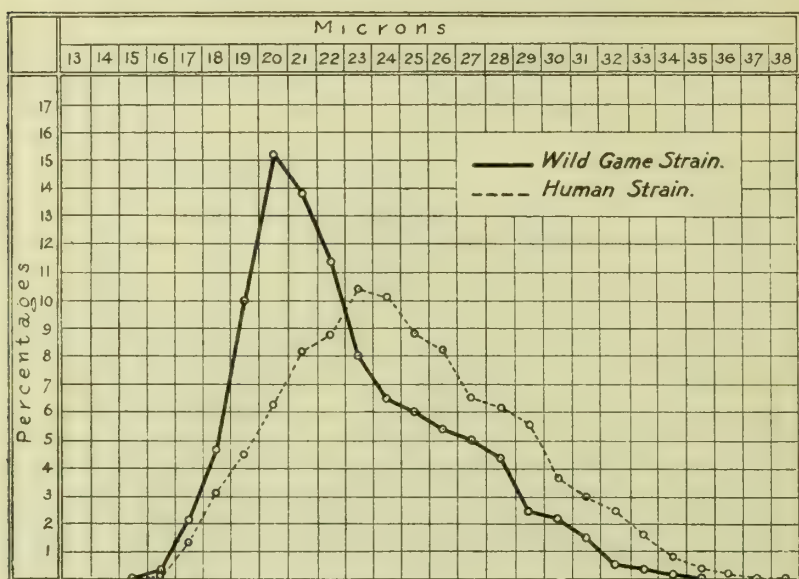


Table XX.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human and Wild-game Strains.

Date.	Strain.	Average, percentage.	Maximum, percentage.	Minimum, percentage.
1912	Human	21.1	52.0	2.0
1912	Wild-game	26.2	33.4	8.4

Conclusions.

1. The five Wild-game strains resemble each other closely, and all belong to the same species of trypanosome.
2. The Wild-game strains and the Human strains, although they differ to some extent, also belong to the same species.
3. This species is *T. rhodesiense* (Stephens and Fantham).
4. There is some reason for the belief that *T. rhodesiense* and *T. brucei* (Plimmer and Bradford) are one and the same species.

VII.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND.—THE WILD *GLOSSINA MORSITANS* STRAIN.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; and Lady BRUCE, R.R.C.

(Received February 24, 1913.)

INTRODUCTION.

These strains were obtained by bringing tsetse flies (*Glossina morsitans*) to the laboratory from the neighbouring "fly-country" and at once allowing them to feed on healthy animals. The term "wild" is used to distinguish these caught flies from flies bred in the laboratory. The first strain was obtained by feeding the flies on a monkey, the remaining four by feeding on dogs. As soon as the healthy animal was found to be infected other animals were inoculated from it. But, as in the case of the Wild-game strains, only trypanosomes from a single rat were used for purposes of measurement and comparison.

I. Morphology of Strain I, Wild *Glossina morsitans*.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest.

Table I.—Measurements of the Length of the Trypanosome of Strain I, Wild *Glossina morsitans*.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·7	34·0	16·0

Table II.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain I, Wild *Glossina morsitans*.

		In microns.									
		16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Total	...	3	11	25	56	62	75	53	31	28	44
Percentages...		0·6	2·2	5·0	11·2	12·4	15·0	10·6	6·2	5·6	8·8

* Reprinted from the Proceedings of the Royal Society, B, Vol. 86.

Table II.—continued.

		In microns.								
		26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	...	16	23	26	22	12	6	4	1	2
Percentages...		3·2	4·6	5·2	4·4	2·4	1·2	0·8	0·2	0·4

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain I, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 655.

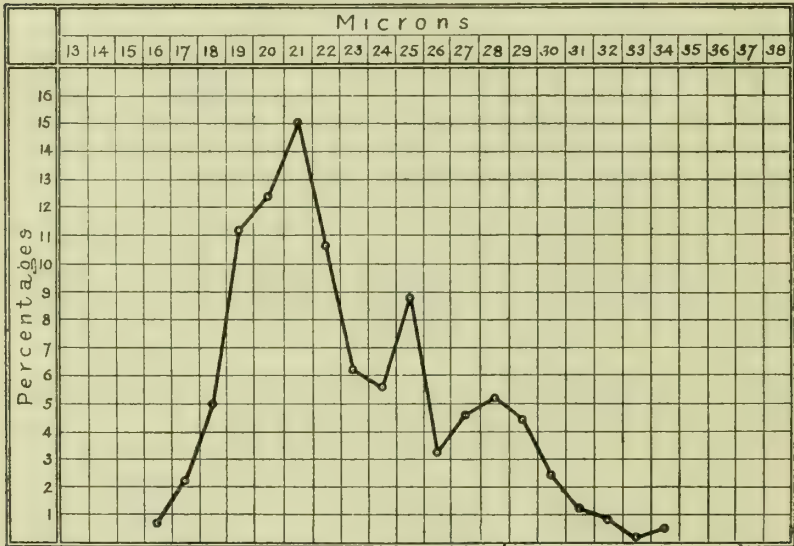


Table III.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain I, Wild *Glossina morsitans*.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 18	655	Rat	6
" 19	655	"	10
" 20	655	"	11
" 21	655	"	17
" 22	655	"	0
" 24	655	"	16
" 25	655	"	28
" 26	655	"	26
" 27	655	"	23
" 28	655	"	0
" 29	655	"	7
" 30	655	"	4
Average			12·3

II. *Morphology of Strain II, Wild Glossina morsitans.*

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest.

Table IV.—Measurements of the Length of the Trypanosome of Strain II, Wild *Glossina morsitans*.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	24·5	34·0	17·0

Table V.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain II, Wild *Glossina morsitans*.

	In microns.									
	17.	18.	19.	20.	21.	22.	23.	24.	25.	
Total ...	1	4	20	43	67	44	48	34	42	
Percentages...	0·2	0·8	4·0	8·6	13·4	8·8	9·6	6·8	8·4	

	In microns.									
	26.	27.	28.	29.	30.	31.	32.	33.	34.	
Total ...	35	33	43	28	23	19	13	2	1	
Percentages...	7·0	6·6	8·6	5·6	4·6	3·8	2·6	0·4	0·2	

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 656.

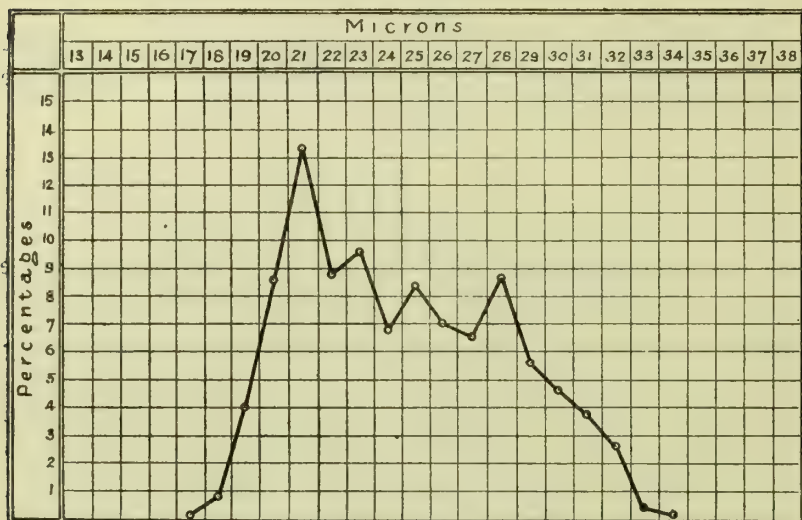


Table VI.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain II, Wild *Glossina morsitans*.

Date.				Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.						
June	17	...	...	656	Rat	8
"	18	...	...	656	"	12
"	19	...	...	656	"	13
"	20	...	...	656	"	22
"	21	...	...	656	"	43
"	22	...	...	656	"	44
"	24	...	...	656	"	27
"	25	...	...	656	"	28
"	26	...	...	656	"	45
"	27	...	...	656	"	47
Average						28.9

III. Morphology of Strain III, Wild *Glossina morsitans*.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest.

Table VII.—Measurements of the Length of the Trypanosome of Strain III, Wild *Glossina morsitans*.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	20·5	31·0	16·0

Table VIII.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain III, Wild *Glossina morsitans*.

	In microns.							
	16.	17.	18.	19.	20.	21.	22.	23.
Total	19	72	84	85	44	24	40	33
Percentages	3·8	14·4	16·8	17·0	8·8	4·8	8·0	6·6

	In microns.							
	24.	25.	26.	27.	28.	29.	30.	31.
Total	27	18	21	12	11	3	4	3
Percentages	5·4	3·6	4·2	2·4	2·2	0·6	0·8	0·6

CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 657.

Table IX.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain III, Wild *Glossina morsitans*.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 17	657	Rat	4
" 18	657	"	9
" 19	657	"	13
" 20	657	"	4
" 21	657	"	2
" 22	657	"	15
" 24	657	"	32
" 25	657	"	18
" 26	657	"	4
" 27	657	"	27
Average			12.8

IV. Morphology of Strain IV, Wild *Glossina morsitans*.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table X.—Measurements of the Length of the Trypanosome of Strain IV, Wild *Glossina morsitans*.

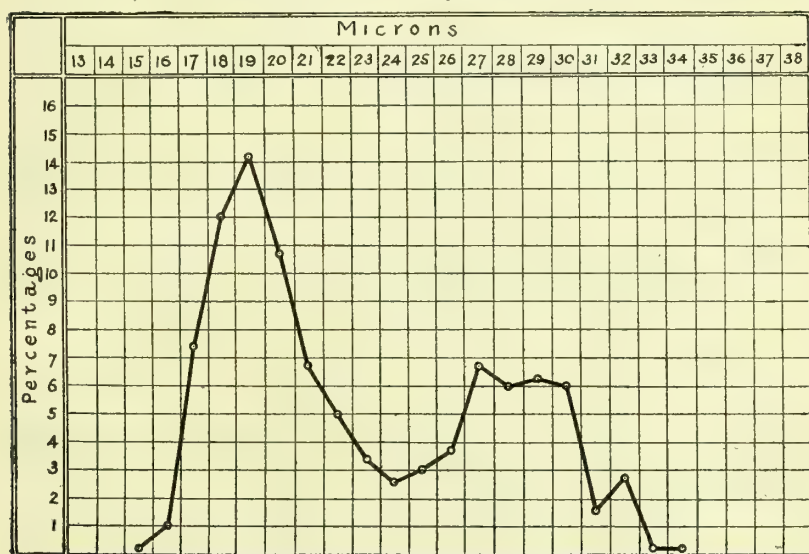
Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22.7	34.0	15.0

Table XI.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain IV, Wild *Glossina morsitans*.

		In microns.									
		15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	...	1	5	37	60	71	54	34	25	17	13
Percentages...		0.2	1.0	7.4	12.0	14.2	10.8	6.8	5.0	3.4	2.6

Table XI—continued.

		In microns.									
		25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	...	15	19	34	30	31	30	8	14	1	1
Percentages...		3.0	3.8	6.8	6.0	6.2	6.0	1.6	2.8	0.2	0.2

CHART 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain IV, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 658.Table XII.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IV, Wild *Glossina morsitans*.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 17	...	...	...
June 18	...	...	...
June 19	...	...	...
June 21	...	...	...
June 22	...	...	...
June 24	...	...	...
June 25	...	...	...
June 26	...	...	...
June 27	...	...	...
June 28	...	...	...
June 29	...	...	...
July 1	...	...	...
July 2	...	...	...
July 3	...	...	...
Average			4.5

V. Morphology of Strain V, Wild *Glossina morsitans*.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table XIII.—Measurements of the Length of the Trypanosome of Strain V, Wild *Glossina morsitans*.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·8	35·0	15·0

Table XIV.—Distribution, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Wild *Glossina morsitans*.

	In microns.									
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total ...	6	4	27	57	94	49	37	22	14	13
Percentages.	1·2	0·8	5·4	11·4	18·8	9·8	7·4	4·4	2·8	2·6

	In microns.											
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	
Total ...	11	19	25	23	29	27	18	13	7	3	2	
Percentages.	2·2	3·8	5·0	4·6	5·8	5·4	3·6	2·6	1·4	0·6	0·4	

CHART 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 660.

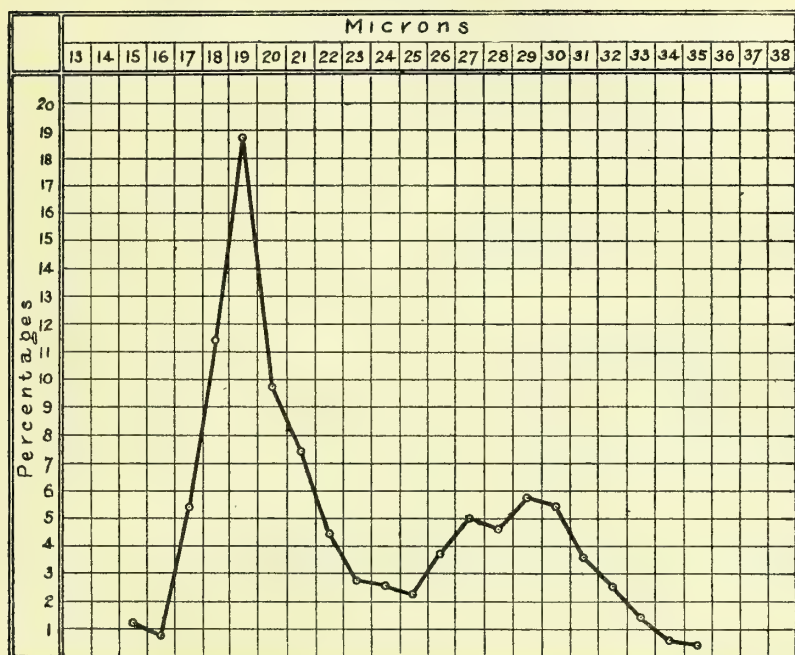


Table XV.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain V, Wild *Glossina morsitans*.

Date.				Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.						
June	18	...	...	660	Rat	0
"	19	...	...	660	"	0
"	20	...	...	660	"	0
"	21	...	...	660	"	8
"	22	...	...	660	"	19
"	24	...	...	660	"	0
"	25	...	...	660	"	6
"	26	...	...	660	"	4
"	27	...	...	660	"	6
"	28	...	...	660	"	6
"	29	...	...	660	"	2
July	2	...	...	660	"	2
"	3	...	...	660	"	1
Average						4.2

Comparison of the Wild Glossina morsitans Strains with one another.

Table XVI.—Measurements of the Length of the Trypanosome of the Wild *Glossina morsitans* Strains.

Date.	Expt. No.	Animal.	Number measured.	From what animals.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	523	Monkey	500	Rat	22·7	34·0	16·0
1912	542	Dog	500	"	24·5	34·0	17·0
1912	551	"	500	"	20·5	31·0	16·0
1912	595	"	500	"	22·7	34·0	15·0
1912	602	"	500	"	22·8	35·0	15·0
			2500	Average	22·6	35·0	15·0

Table XVII.—Distribution in respect to Length of 2,500 Individuals of the Trypanosome of the five Wild *Glossina morsitans* Strains.

	In microns.									
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total ...	7	31	148	230	326	252	237	184	143	115
Percentages.	0·3	1·2	5·9	9·2	13·1	10·1	9·5	7·3	5·7	4·6

	In microns.										
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.
Total ...	130	110	127	133	113	96	54	44	11	7	2
Percentages.	5·2	4·4	5·1	5·3	4·5	3·8	2·2	1·8	0·4	0·3	0·1

CHART 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 2,500 Individuals of the Trypanosome of the Wild *Glossina morsitans* Strains.

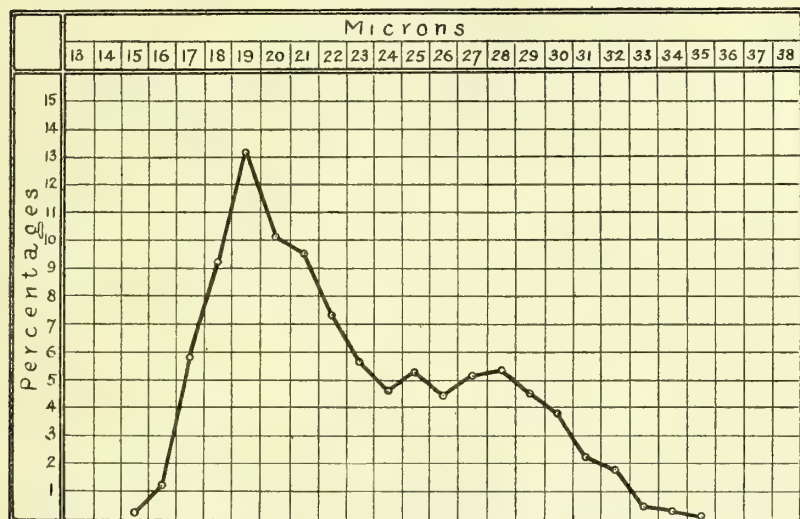


Table XVIII.—Comparison of the Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the five Wild *Glossina morsitans* Strains.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912	523	Monkey	12.3
1912	542	Dog	28.9
1912	551	"	12.8
1912	595	"	4.5
1912	602	"	4.2
Average			12.5

Comparison of the Human Strain with the Wild-game and Wild *Glossina morsitans* Strains.

Table XIX.—Average Length of the Trypanosome of the Human, Wild-game, and Wild *Glossina morsitans* Strains.

Strain.	Number of trypanosomes measured.	Animal.	In microns.		
			Average length.	Maximum length.	Minimum length.
Human	3600	Rat	24.2	38.0	15.0
Wild-game	2500	"	22.6	35.0	15.0
Wild <i>G. morsitans</i>	2500	"	22.6	35.0	15.0

It is remarkable that the Wild-game strains and the Wild *Glossina morsitans* strains should come out exactly alike. Why the Human strains differ to some extent from the other two cannot at present be explained.

The curves of the Wild-game strains and the Wild *Glossina morsitans* strains are so similar that there can be little doubt the same species of trypanosome is being dealt with. The Human strain differs so much, that the suspicion must present itself that in some way more than one species is being dealt with. The three Human strains which differ most from the Wild-game and Wild *Glossina morsitans* type are Stephens and Fantham's case of Armstrong, in Liverpool; Strain I, Mkan-yanga; and Strain III, Chituluka. On examining these three strains, however, from every possible point of view, nothing, except the difference in the type of curve, can be found to justify this suspicion.

CHART 7.—Curves representing the Distribution, by Percentages, of 3,600 Individuals of the Trypanosome of the Human Strains, 2,500 of the Wild-game Strains, and 2,500 of the Wild *Glossina morsitans* Strains.

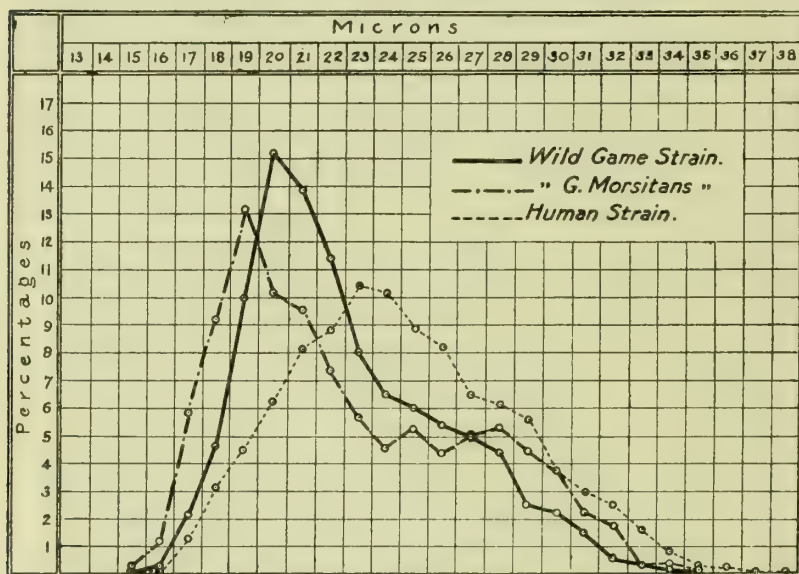


Table XX.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human, Wild-game, and Wild *Glossina morsitans* Strains.

Date.	Strain.	Percentages.		
		Average.	Maximum.	Minimum.
1912	Human	21.1	52.0	2.0
1912	Wild-game	26.2	33.4	8.4
1912	Wild <i>G. morsitans</i> ...	12.5	28.9	4.2

Conclusions.

1. The five Wild *Glossina morsitans* strains resemble each other closely, and all belong to the same species of trypanosome.
 2. The Wild *Glossina morsitans* strain, the Human strain, and the Wild-game strain, belong to the same species.
 3. This species is *T. rhodesiense* (Stephens and Fantham).
 4. It is probable that *T. rhodesiense* and *T. brucei* (Plimmer and Bradford) are identical.
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VIII. INFECTIVITY OF *GLOSSINA MORSITANS*
IN NYASALAND.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; and Lady BRUCE, R.R.C.

(Received February 24, 1913.)

INTRODUCTION.

The object of this paper is to put on record the proportion of tsetse flies found in this district of Nyasaland to be infected with disease-producing trypanosomes, and, further, to identify the species of trypanosomes with which the flies are infected.

It must be understood that this paper only deals with the district lying near the camp at Kasu and not to every part of Nyasaland. The geographical position and other features of this district have already been described in a previous paper.† It is known as the "Sleeping Sickness Area" or "Proclaimed Area," and in it almost all the cases of the Human Trypanosome Disease of Nyasaland have occurred. This disease is caused by *Trypanosoma rhodesiense*, and the natives give it the name of "Kaodzera," but there is a good deal of evidence accumulating that *T. rhodesiense* is in reality *T. brucei*, in which case the disease would be known as "Nagana."

Definitions.

In this paper an *infective* fly is one which contains trypanosomes which have reached the final stage of their development and are capable of giving rise to disease. An *infective* fly is one in which the development of the trypanosomes may not have reached this final stage, and where, therefore, it may not as yet be disease-producing. An *infective* fly must be infected, whereas an *infected* fly need not be *infective*.

* Reprinted from the Proceedings of the Royal Society, B, Vol. 86.

† *Supra*, p. 16.

“Fly” means the tsetse fly, and in this paper the species dealt with is *Glossina morsitans*.

Methods Employed.

The method employed in studying the infectivity of the flies was simple. Native boys were employed in catching the flies, which were brought up to Kasu in small cages by a native cyclist. Each cage of flies was fed on three healthy animals—the first day on a monkey, the second on a dog, and the third on a goat. To ensure, as far as possible, that each animal was fed on by every fly, the flies were fed nine times—three times on each animal.

The number of flies brought up each day would probably average about 60, and as each animal was fed on by three cagefuls, then each monkey, dog, and goat ran the gauntlet of some 180 flies. It is therefore impossible to arrive at any very precise knowledge of the proportion of infective flies in each cage.

Infectivity of the Flies.

As will be seen from Table I, every experiment, with the exception of one, was positive, and on two occasions a goat was infected by all the four species of pathogenic trypanosomes occurring in this neighbourhood.

The Commission showed in a previous paper* that one in three of the wild game found in this district is infected with trypanosomes, and recommended that the animals should be destroyed. If this were done and a year allowed to elapse, the proportion of infective flies then found would be an index of the usefulness or futility of such operations.

The Commission is of opinion that the wild game is the principal factor in the spread of trypanosome disease, and that, for practical purposes, the smaller mammals, birds, and reptiles need not be taken into account.

The following table gives, in the first column, the date the first cageful of flies was fed on the monkey, the second column the number of flies fed; the signs *plus* and *minus* show the result of feeding the flies on the monkey, dog, and goat.

The four species of trypanosomes carried by the “fly” in this district are *T. brucei* vel *rhodesiense*, *T. pecorum*, *T. simiæ*, and *T. capræ*. The first and second of these attack all three animals, the third the monkey and goat, being harmless to the dog, whereas the fourth only produces disease in the goat.

Where no *plus* or *minus* sign occurs it means that an animal was not available. For example, the experiment beginning on January 20 shows that the monkey was infected by *T. simiæ*, the goat by *T. brucei*, and that no dog was available. The experiment on February 21 shows that neither the dog nor goat became infected by the bites of 170 flies, and that no monkey was available.

* *Supra*, p. 24.

Table I.—The Result of Feeding 10,081 Tsetse Flies (*G. morsitans*), caught in the "Proclaimed Area," Nyasaland, on Monkeys, Dogs, and Goats.

1912.	Num- ber of flies fed.	Monkey.					Dog.				Goat.			
		<i>T. brucei</i> vel <i>T. rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simiae</i> .	<i>T. caprae</i> .	<i>T. brucei</i> vel <i>T. rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simiae</i> .	<i>T. caprae</i> .	<i>T. brucei</i> vel <i>T. rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simiae</i> .	<i>T. caprae</i> .	
Jan. 20 ...	296	—	—	+	—					+	—	—	—	
" 24 ...	370	—	—	+	—					—	+	—	+	
" 29 ...	280	—	—	+	—						+	+	—	
Feb. 2 ...	295	—	—	+	—					—	+	+	+	
" 8 ...	220	—	—	+	—					—	+	+	+	
" 13 ...	200	—	+	+	—					—	—	+	+	
" 16 ...	195					+	—	—	—	+	—	—	—	
" 21 ...	170					—	—	—	—	—	—	—	—	
" 26 ...	170					—	—	—	—	—	—	+	—	
Mar. 2 ...	140					—	—	—	—	—	—	—	+	
" 9 ...	165					—	+	—	—	—	—	—	+	
" 14 ...	100					—	—	—	—	—	—	—	+	
" 17 ...	160					—	+	—	—	—	+	—	—	
" 22 ...	205					—	+	—	—	+	+	+	—	
Apr. 3 ...	135					—	+	—	—	—	+	+	+	
" 10 ...	275	+	—	+	—	+	+	—	—	—	—	—	+	
" 15 ...	330	—	—	+	—	—	+	—	—	—	+	+	+	
" 18 ...	200	—	—	+	—	—	+	—	—	—	+	+	+	
" 18 ...	180	—	—	+	—	—	+	—	—	—	—	+	—	
" 23 ...	230	—	—	+	—	—	+	—	—	—	—	—	+	
" 23 ...	140	—	+	—	—	—	+	—	—	—	+	—	—	
" 26 ...	100	—	—	+	—	—	+	—	—	+	+	+	—	
" 27 ...	260	—	—	+	—	—	—	—	—	—	+	+	—	
May 3 ...	155	+	+	+	—	—	+	—	—	—	—	—	+	
" 3 ...	96	—	—	—	—	—	+	—	—	—	+	—	—	
" 8 ...	330	+	—	+	—	+	—	—	—	—	+	—	+	
" 9 ...	120	—	—	+	—	—	+	—	—	—	+	—	+	
" 13 ...	50	—	—	—	—	+	—	—	—	—	—	+	+	
" 14 ...	250	—	—	+	—	—	+	—	—	+	+	+	+	
" 17 ...	190					+	+	—	—	—	—	+	+	
" 24 ...	113					—	+	—	—	—	+	+	+	
" 29 ...	120	—	—	—	—	—	+	—	—	—	+	—	—	
" 29 ...	230	—	—	—	—	—	+	—	—	—	+	—	—	
" 29 ...	320					+	+	—	—	—	+	—	+	
" 29 ...	240	—	+	—	—	—	+	—	—	—	+	+	—	
" 29 ...	100					—	+	—	—	—	+	+	+	
" 31 ...	175	+	—	+	—	+	+	—	—	—	+	+	—	
June 2 ...	300					—	—	—	—	—	—	+	+	
" 6 ...	210	—	+	—	—	—	+	—	—	—	+	—	+	
" 7 ...	230	+	—	—	—	+	+	—	—	+	+	—	—	
" 11 ...	160	—	—	+	—	—	+	—	—	—	+	—	+	
" 18 ...	135	—	—	—	—	—	+	—	—	—	+	—	—	
" 25 ...	90	+	—	—	—	+	—	—	—	—	+	—	+	
July 3 ...	95					—	+	—	—	—	+	—	—	
Sept. 25 ...	70	—	+	—	—									
" 27 ...	25	+	—	—	—									
Oct. 29 ...	87	+	—	—	—	+	—	—	—	+	+	—	+	
Carried forward. }		8	6	19	0	10	30	0	0	7	30	16	29	

Table I—continued.

1912.	Num- ber of flies fed.	Monkey.				Dog.				Goat.			
		<i>T. brucei</i> vel <i>T. rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simia</i> .	<i>T. capræ</i> .	<i>T. brucei</i> vel <i>T. rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simia</i> .	<i>T. capræ</i> .	<i>T. brucei</i> vel <i>T. rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simia</i> .	<i>T. capræ</i> .
Brought forward. }		8	6	19	0	10	30	0	0	7	30	16	29
Nov. 5 ...	145	—	—	—	—	—	—	—	—	—	—	—	+
" 11 ...	150	—	—	+	—	—	+	—	—	—	+	—	+
" 18 ...	157	—	—	+	—	—	+	—	—	—	—	+	+
" 21 ...	95	—	—	—	—	+	—	—	—	+	—	—	—
" 25 ...	180	—	—	+	—	+	—	—	—	+	+	—	—
Dec. 3 ...	180	—	+	+	—	—	+	—	—	—	+	—	+
" 6 ...	198	+	+	+	—	+	—	—	—	+	—	—	+
" 11 ...	156	—	—	+	—	+	—	—	—	+	+	—	—
" 16 ...	113	—	+	+	—	—	+	—	—	—	+	—	+
Total ...	10,081	9	9	26		14	34			11	35	17	35

Table II.—The Number of Times a Monkey, Dog, and Goat became infected with *Trypanosoma brucei* vel *rhodesiense*, *T. pecorum*, *T. simia*, and *T. capræ* in a Series of 56 Experiments, averaging 180 Tsetse Flies each.

<i>T. brucei</i> vel <i>rhodesiense</i> .			<i>T. pecorum</i> .			<i>T. simia</i> .			<i>T. capræ</i> .		
Monkey.	Dog.	Goat.	Monkey.	Dog.	Goat.	Monkey.	Dog.	Goat.	Monkey.	Dog.	Goat.
9	14	11	9	34	35	26	0	17	0	0	35

This shows that the monkey is less susceptible to *T. brucei* and *T. pecorum* than the dog, whereas it is remarkably so to *T. simia*. The dog is not susceptible to *T. simia*, and neither the monkey nor dog to *T. capræ*.

Table III.—The Proportion per 1,000 Tsetse Flies, caught in the "Sleeping Sickness" Area of Nyasaland, found to be Infective with Pathogenic Trypanosomes.

<i>T. brucei</i> vel <i>rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simia</i> .	<i>T. capræ</i> .
Per 1,000. 2.0	Per 1,000. 4.6	Per 1,000. 3.4	Per 1,000. 3.5

This is only allowing one infective fly to each series of flies fed on the experimental animals, and is therefore the irreducible minimum. The average number of flies fed on each animal was 180, and it might well be that there were present in the same batch several flies infective with the same species of trypanosome. Ten thousand flies gave rise to 135 infections, and taking it for granted that no fly was infective with more than one species of trypanosome, then 13·5 per 1,000 flies are infective with one or other of the disease-producing trypanosomes of this district.

Table IV.—Number of Times the Species of Trypanosomes under consideration were found in 56 Experiments.

<i>T. brucei</i> vel <i>rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simiæ</i> .	<i>T. capræ</i> .
20	46	34	35
35·7 per cent.	82·1 per cent.	60·7 per cent.	62·5 per cent.

This means that in experiments carried out in the manner described *T. brucei* may be expected to turn up once in every three series, *T. pecorum* eight times in ten, and *T. simiæ* and *T. capræ* six times in ten.

Months and Seasons.

On examining Table I. it will be seen that these infective flies occur all the year round, and are just as numerous during one season as another. It will also be seen that no experiments on the infectivity of the flies were carried out during July and August. This was due to the fact that all the energy of the Commission was devoted during these two months to the study of the wild game.

Conclusions.

1. The tsetse flies (*Glossina morsitans*) caught in the "fly-country" near Kasu are infected with four species of disease-producing trypanosomes—*T. brucei* vel *rhodesiense*, *T. pecorum*, *T. simiæ*, and *T. capræ*.
2. The proportion of infective flies is 13·5 per 1,000.
3. The proportion of flies infective with *T. brucei* vel *rhodesiense*, the cause of the Human Trypanosome Disease of Nyasaland, is 2 per 1,000.
4. The flies are found infective all the year round.
5. To prevent the infection of tsetse flies it is proposed that the experiment should be tried of destroying all the wild game in the "Proclaimed Area" of Nyasaland.

IX.—TRYPANOSOME DISEASES OF DOMESTIC ANIMALS
IN NYASALAND. III.—*TRYPANOSOMA PECORUM*.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; and Lady BRUCE, R.R.C.

(Received April 14, 1913.)

INTRODUCTION.

This trypanosome has been found in the neighbourhood of the camp at Kasu, in cattle, wild game and wild tsetse flies. In a herd of cattle belonging to the Mvera Mission, which lies about two miles to the east, 32 per cent. were found to be infected by this disease. The mission station is built near the edge of the "fly-country," and there is little doubt that the cattle were at times exposed to the bite of the "fly." After the disease had been discovered to be present in the herd the animals were prevented from grazing in the direction of the danger, and since then no new cases have occurred. It is also the species of trypanosome most commonly found in the blood of the wild game in this district, and consequently the tsetse fly is found infected with it more frequently than with any other. It is one of the most important trypanosome diseases of domestic animals in Central Africa, as it affects them all—horses, cattle, goats, sheep, pigs, and dogs.

MORPHOLOGY OF *TRYPANOSOMA PECORUM*.

The description already given† of this species of trypanosome as regards its movements and appearance when alive, its shape, contents of cell, etc., when stained, are applicable to the species as it occurs in Nyasaland and need not be repeated.

I. *The Mvera Cattle Strain.*

Length.—The following table gives the length of this trypanosome as found in the donkey, ox, goat, dog, and rat—500 trypanosomes in all:—

* Reprinted from the Proceedings of the Royal Society, B, Vol. 87.

† 'Roy. Soc. Proc.,' 1910, B, Vol. 82, p. 468. (These Reports No. XI., p. 128.)

Table I.—Measurements of the Length of the Trypanosome of the Mvera Cattle Strain.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Jan. 25	70	Donkey	Osmic acid	Giemsa	14·1	16·0	11·0
March 15	291	"	"	"	12·9	16·0	11·0
April 16	445	"	"	"	14·0	16·0	12·0
" 18	291	"	"	"	13·0	15·0	12·0
Jan. 17	102	Ox ...	"	"	14·8	17·0	13·0
" 17	103	" ...	"	"	13·9	16·0	12·0
Feb. 9	100	" ...	"	"	13·0	15·0	11·0
" 9	103	" ...	"	"	14·6	17·0	11·0
" 9	103	" ...	"	"	14·5	17·0	12·0
" 20	104	" ...	"	"	14·0	16·0	12·0
" 26	100	" ...	"	"	14·4	16·0	11·0
March 1	227	" ...	"	"	14·0	16·0	12·0
" 1	228	" ...	"	"	14·5	18·0	11·0
Feb. 26	208	Goat ...	"	"	13·2	15·0	9·0
" 29	178	" ...	"	"	13·2	15·0	10·0
March 11	206	" ...	"	"	14·6	17·0	11·0
" 11	208	" ...	"	"	14·6	16·0	12·0
May 20	199	" ...	"	"	13·3	15·0	12·0
Feb. 15	16	Dog ...	"	"	13·8	16·0	11·0
" 26	212	" ...	"	"	14·4	16·0	13·0
" 26	214	" ...	"	"	13·3	16·0	11·0
" 26	217	" ...	"	"	13·5	15·0	12·0
March 4	218	" ...	"	"	13·8	16·0	12·0
" 14	223	Rat ...	"	"	14·8	16·0	13·0
" 18	223	" ...	"	"	14·7	16·0	13·0
					14·0	18·0	9·0

The average length of the trypanosome of the Mvera Cattle strain, in different species of animals, is as follows (Table II.):—

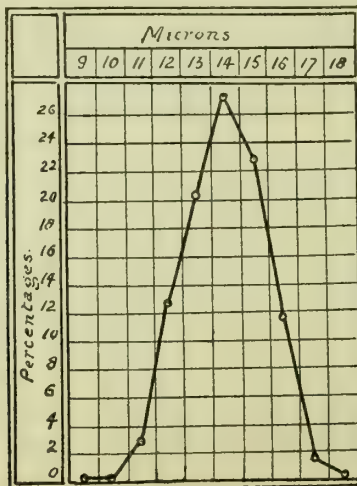
Rats were, as a rule, immune to this strain, but on one occasion, in a case of mixed infection, it was found along with *T. brucei*. It was thought that the presence of *T. brucei* may have broken down the rat's immunity to *T. pecorum*.

Table II.

Species of Animal.			Number of trypanosomes measured.	In microns.		
				Average length.	Maximum length.	Minimum length.
Donkey	...	...	80	13·5	16·0	11·0
Ox	...	...	180	14·2	17·0	11·0
Goat	...	...	100	13·8	17·0	9·0
Dog	...	...	100	13·8	16·0	11·0
Rat	...	...	40	14·8	16·0	13·0

Table III.—Distribution in respect to Length of 500 Individuals of the Mvera Cattle Strain.

Animal.	In microns.											Average length.
	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	
Donkey ...	—	—	1	2	5	2	6	4	—	—	—	14.1
" ...	—	—	1	8	5	4	1	1	—	—	—	12.9
" ...	—	—	—	2	4	8	4	2	—	—	—	14.0
" ...	—	—	—	9	3	6	2	—	—	—	—	13.0
Ox ...	—	—	—	—	6	4	7	2	1	—	—	14.8
" ...	—	—	—	4	5	3	4	4	—	—	—	13.9
" ...	—	—	3	4	6	3	4	—	—	—	—	13.0
" ...	—	—	2	1	1	6	5	2	3	—	—	14.6
" ...	—	—	—	1	2	8	5	3	1	—	—	14.5
" ...	—	—	—	2	5	5	7	1	—	—	—	14.0
" ...	—	—	1	2	1	5	5	6	—	—	—	14.4
" ...	—	—	—	3	4	5	6	2	—	—	—	14.0
" ...	—	—	1	1	3	5	6	1	2	1	—	14.5
Goat ...	1	—	—	4	5	7	3	—	—	—	—	13.2
" ...	—	1	2	1	8	4	4	—	—	—	—	13.2
" ...	—	—	—	1	4	4	4	7	—	—	—	14.6
" ...	—	—	1	2	1	4	5	6	1	—	—	14.6
" ...	—	—	—	6	4	7	3	—	—	—	—	13.3
Dog ...	—	—	1	4	2	6	5	2	—	—	—	13.8
" ...	—	—	—	4	6	7	3	3	—	—	—	14.4
" ...	—	—	2	3	5	7	2	1	—	—	—	13.3
" ...	—	—	—	2	9	6	3	—	—	—	—	13.5
" ...	—	—	—	2	7	5	4	2	—	—	—	13.8
Rat ...	—	—	—	—	1	7	7	5	—	—	—	14.8
" ...	—	—	—	—	1	9	5	5	—	—	—	14.7
Totals ...	1	1	15	64	101	136	114	59	8	1	—	
Percentages...	0.2	0.2	3.0	12.8	20.2	27.2	22.8	11.8	1.6	0.2	—	

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *Trypanosoma pecorum*—The Mvera Cattle Strain.

This curve is made up of measurements from 80 specimens of trypanosomes taken from the donkey, 180 from the ox, 100 from the goat, 100 from the dog, and 40 from the rat.

Breadth.—Measured across the broadest part of the body, but not including the undulating membrane, the Mvera Cattle strain of *T. pecorum* averages 1·97 microns (maximum 3, minimum 1·25).

II. The Wild-game Strain.

The citrated blood of the wild game was brought up from the "fly-country" by a motor-cyclist, and at once injected into a goat, monkey and dog. A description of the kind of wild game found in this locality and the trypanosomes they are infected with was given in a previous paper.*

Length.—The following table gives the length of this trypanosome as found in the goat, monkey, dog, and rat, 500 trypanosomes in all:—

Table IV.—Measurements of the Length of *Trypanosoma pecorum*—The Wild-game Strain.

Date.	No. of experi- ment.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maxi- mum length.	Mini- mum length.
1912.							
Feb. 1 ...	45	Goat	Osmic acid	Giemsa	15·7	17·0	13·0
Aug. 26 ...	898	"	"	"	14·6	17·0	12·0
Sept. 2 ...	1031	"	"	"	14·1	16·0	12·0
" 9 ...	1037	"	"	"	13·9	16·0	12·0
" 9 ...	1039	"	"	"	15·0	18·0	12·0
" 9 ...	1121	"	"	"	14·5	16·0	13·0
" 12 ...	1039	"	"	"	14·3	16·0	12·0
" 23 ...	1383	"	"	"	13·3	15·0	11·0
Oct. 7 ...	1307	"	"	"	13·7	16·0	12·0
" 7 ...	1383	"	"	"	13·4	16·0	12·0
April 22 ...	403	Monkey	"	"	14·1	17·0	12·0
Sept. 9 ...	1085	"	"	"	14·6	17·0	11·0
April 8 ...	355	Dog	"	"	14·2	17·0	12·0
" 11 ...	356	"	"	"	14·3	17·0	12·0
Aug. 19 ...	1015	"	"	"	14·8	17·0	13·0
Sept. 2 ...	1086	"	"	"	14·3	16·0	12·0
" 9 ...	1086	"	"	"	14·9	17·0	13·0
" 12 ...	1015	"	"	"	14·0	16·0	12·0
" 12 ...	1086	"	"	"	14·1	16·0	13·0
Nov. 7 ...	1510	"	"	"	14·0	16·0	12·0
" 18 ...	1510	"	"	"	14·2	16·0	12·0
May 16 ...	511	Rat	"	"	14·3	18·0	12·0
Nov. 26 ...	1518	"	"	"	14·1	16·0	12·0
Dec. 26 ...	1608	"	"	"	15·0	18·0	13·0
" 26 ...	1608	"	"	"	14·6	17·0	13·0
					14·3	18·0	11·0

* 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 269. (*Supra*, p. 16.)

The average length of the trypanosome of the Wild-game strain, in different species of animals, is as follows:—

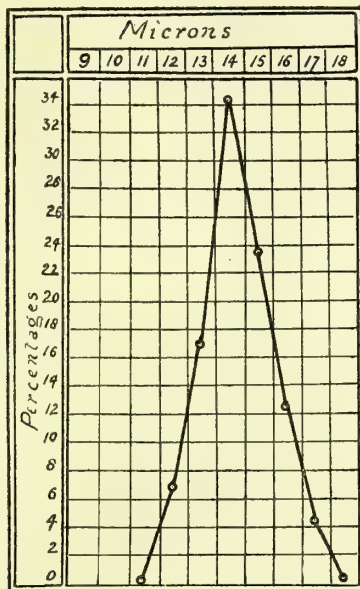
Table V.

Species of animals.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	200	14·2	18·0	11·0
Monkey	40	14·3	17·0	11·0
Dog	180	14·3	17·0	12·0
Rat	80	14·5	18·0	12·0

Table VI.—Distribution in respect to Length of 500 Individuals of the Wild-game Strain.

Animal.	In microns.								Average length.
	11.	12.	13.	14.	15.	16.	17.	18.	
Goat	—	—	1	4	6	5	4	—	15·7
"	—	1	2	7	6	2	2	—	14·6
"	—	1	3	11	3	2	—	—	14·1
"	—	1	8	5	3	3	—	—	13·9
"	—	1	2	3	7	4	2	1	15·0
"	—	—	4	7	4	5	—	—	14·5
"	—	2	—	10	5	3	—	—	14·3
"	1	4	4	10	1	—	—	—	13·3
"	—	1	8	8	2	1	—	—	13·7
"	—	5	5	8	1	1	—	—	13·4
Monkey	—	3	3	7	4	1	2	—	14·1
"	1	—	3	7	3	3	3	—	14·6
Dog	—	3	2	5	8	1	1	—	14·2
"	—	2	6	4	2	4	2	—	14·3
"	—	—	4	4	5	5	2	—	14·8
"	—	1	1	11	5	2	—	—	14·3
"	—	—	1	6	8	4	1	—	14·9
"	—	3	3	5	8	1	—	—	14·0
"	—	—	5	9	4	2	—	—	14·1
"	—	2	5	5	7	1	—	—	14·0
"	—	1	4	8	3	4	—	—	14·2
Rat	—	1	2	9	7	—	—	1	14·3
"	—	2	3	8	5	2	—	—	14·1
"	—	—	2	6	5	5	1	1	15·0
"	—	—	4	5	7	2	2	—	14·6
Total	2	34	85	172	119	63	22	3	
Percentages	0·4	6·8	17·0	34·4	23·8	12·6	4·4	0·6	

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. pecorum*, Wild-game Strain, taken at random from various animals.

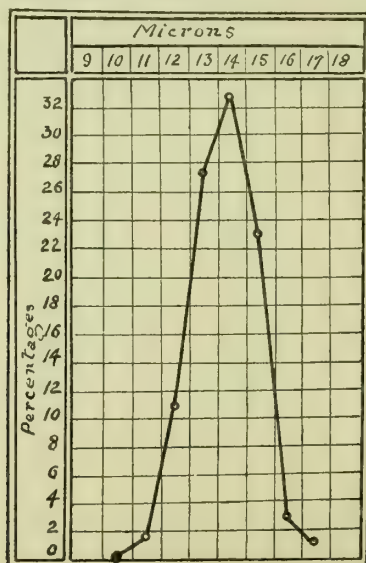


This curve is made up of measurements from 200 specimens of trypanosomes taken from the goat, 40 from the monkey, 180 from the dog, and 80 from the rat.

The following table gives the distribution in respect to length of *T. pecorum*, Wild-game strain, taken from a single rat on consecutive days, as was done in the description of the morphology of *T. brucei* vel *rhodesiense*. In this case the rat is not a suitable animal, since many strains of *T. pecorum* have no effect on it:—

Table VII.—Distribution in respect to Length of 500 Individuals of the Wild-game Strain, taken on nine consecutive days from Rat 510.

Animal.	In microns.								Average length.
	10.	11.	12.	13.	14.	15.	16.	17.	
Rat	—	—	2	2	7	6	2	1	14.3
"	—	—	4	4	11	1	—	—	13.4
"	—	1	1	8	8	2	—	—	13.4
"	—	—	3	10	3	4	—	—	13.4
"	—	1	1	9	6	3	—	—	13.4
"	—	—	6	3	8	3	—	—	13.4
"	—	—	1	8	6	4	1	—	13.8
"	—	—	1	5	6	7	1	—	14.1
"	—	—	2	7	4	7	—	—	13.8
"	—	—	—	3	5	11	—	1	14.5
"	—	2	1	4	9	4	—	—	13.6
"	—	—	7	7	5	1	—	—	13.0
"	—	—	5	4	9	1	1	—	13.4
"	—	1	1	8	7	3	—	—	13.5
"	1	—	3	8	3	5	—	—	13.3
"	—	1	—	2	5	5	5	2	14.8
"	—	—	—	10	5	5	—	—	13.7
"	—	1	2	4	8	5	—	—	13.7
"	—	—	1	3	8	7	—	1	14.2
"	—	—	1	6	9	4	—	—	13.8
"	—	—	—	2	7	8	3	—	14.6
"	—	2	1	5	4	8	—	—	13.7
"	—	—	5	4	9	2	—	—	13.4
"	—	—	3	6	5	6	—	—	13.7
"	—	—	2	5	6	5	1	1	14.0
Total ...	1	9	53	137	163	117	14	6	
Percentages ...	0.2	1.8	10.6	27.4	32.6	23.4	2.8	1.2	

CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. pecorum*, Wild-game Strain, taken on nine consecutive days from Rat 510.

Breadth.—*T. pecorum*, Wild-game strain, measured across the broadest part of the body, not including the undulating membrane, averages 1·97 micron (maximum 2·75, minimum 1·25).

III. *The Wild Glossina morsitans Strain.*

The tsetse flies were brought from the neighbouring "fly-country" and fed on goats, monkeys, and dogs as described in a former paper.*

Length.—The following table gives the length of this trypanosome as found in the goat, monkey, dog, guinea-pig, and rat, 500 trypanosomes in all:—

Table VIII.—Measurements of the Length of *T. pecorum*—
The Wild *Glossina morsitans* Strain.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Feb. 15	89	Goat ...	Osmic acid	Giemsa	12·5	14·0	10·0
" 19	89	" ...	"	"	13·9	17·0	11·0
Mar. 4	89	" ...	"	"	14·2	16·0	12·0
April 25	363	" ...	"	"	14·4	17·0	11·0
June 13	422	" ...	"	"	13·3	16·0	10·0
" 17	422	" ...	"	"	13·6	16·8	11·0
" 20	564	" ...	"	"	13·0	15·0	10·0
" 24	565	" ...	"	"	13·3	15·0	11·0
July 1	565	" ...	"	"	12·9	15·0	9·0
" 8	634	" ...	"	"	14·5	17·0	12·0
June 10	450	Monkey...	"	"	13·6	15·0	12·0
July 8	623	" ...	"	"	13·7	16·0	11·0
Mar. 9	258	Dog ...	"	"	14·5	17·0	12·0
" 18	256	" ...	"	"	13·5	16·0	11·0
" 18	258	" ...	"	"	13·7	15·0	12·0
" 18	322	" ...	"	"	13·4	15·0	11·0
" 21	256	" ...	"	"	14·6	17·0	11·0
" 21	258	" ...	"	"	14·1	17·0	12·0
" 21	322	" ...	"	"	15·1	17·0	12·0
April 22	400	" ...	"	"	14·7	17·0	13·0
May 16	452	Guinea-pig	"	"	14·6	18·0	12·0
" 9	454	Rat ...	"	"	13·9	17·0	12·0
" 9	455	" ...	"	"	14·0	16·0	12·0
" 16	454	" ...	"	"	13·7	16·0	12·0
" 16	455	" ...	"	"	14·3	16·0	10·0
					13·9	18·0	9·0

* 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 408.

The average length of the trypanosome of the Wild *Glossina morsitans* strain, in different species of animals, is as follows:—

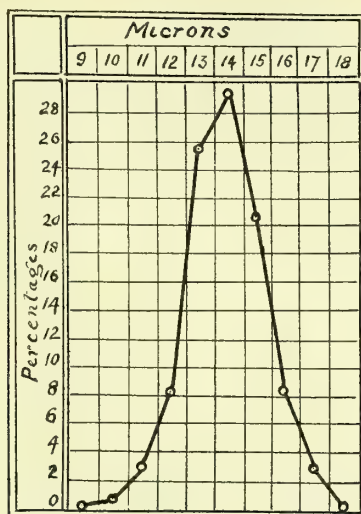
Table IX.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	200	13·5	17·0	9·0
Monkey	40	13·6	16·0	11·0
Dog	160	14·2	17·0	11·0
Guinea-pig	20	14·6	18·0	12·0
Rat	80	14·0	17·0	10·0

Table X.—Distribution in respect to Length of the Wild *Glossina morsitans* Strain.

Animal.	In microns.										Average length.
	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	
Goat	—	1	3	5	6	5	—	—	—	—	12·5
"	—	—	1	4	3	5	3	3	1	—	13·9
"	—	—	—	2	1	9	6	2	—	—	14·2
"	—	—	2	—	3	4	7	2	2	—	14·4
"	—	1	3	1	3	9	2	1	—	—	13·3
"	—	—	1	—	9	7	2	1	—	—	13·6
"	—	1	—	4	8	6	1	—	—	—	13·0
"	—	—	1	1	11	4	3	—	—	—	13·3
"	1	—	1	3	8	6	1	—	—	—	12·9
"	—	—	—	1	3	5	7	3	1	—	14·5
Monkey	—	—	—	2	6	10	2	—	—	—	13·6
"	—	—	1	1	8	4	4	2	—	—	13·7
Dog	—	—	—	1	3	6	6	3	1	—	14·5
"	—	—	1	2	8	5	2	2	—	—	13·5
"	—	—	—	3	4	9	4	—	—	—	13·7
"	—	—	1	3	8	4	4	—	—	—	13·4
"	—	—	1	2	2	4	3	5	3	—	14·6
"	—	—	—	2	5	5	5	2	1	—	14·1
"	—	—	—	1	—	3	10	4	2	—	15·1
"	—	—	—	—	4	5	6	3	2	—	14·7
Guinea-pig	—	—	—	1	4	4	6	3	1	1	14·6
Rat	—	—	—	1	7	7	3	1	1	—	13·9
"	—	—	—	1	5	7	6	1	—	—	14·0
"	—	—	—	1	9	5	4	1	—	—	13·7
"	—	1	—	—	1	9	6	3	—	—	14·3
Total	1	4	16	42	129	147	103	42	15	1	
Percentages ...	0·2	0·8	3·2	8·4	25·8	29·4	20·6	8·4	3·0	0·2	

CHART 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. pecorum*—The Wild *Glossina morsitans* Strain.



This curve is made up of measurements from 200 specimens of trypanosomes taken from the goat, 40 from the monkey, 160 from the dog, 20 from the guinea-pig, and 80 from the rat.

Breadth.—Measured across the broadest part of the body, not including the undulating membrane, *T. pecorum*, Wild *Glossina morsitans* strain, averages 1.94 micron (maximum 2.75, minimum 1.25).

COMPARISON OF THE MVERA CATTLE STRAIN, WILD-GAME STRAIN, AND WILD GLOSSINA MORSITANS STRAIN OF *T. PECORUM*.

The following table gives the average length of this trypanosome in the three strains under consideration, as found in the donkey, ox, goat, monkey, dog, guinea-pig, and rat:—

Table XI.—Measurements of the Length of the Trypanosomes of the Three Strains.

Date.	Strain.	No. of trypanosomes measured.	Animal.	In microns.		
				Average length.	Maximum length.	Minimum length.
1912	Mvera cattle ...	500	Various	14.0	18.0	9.0
1912	Wild-game ...	500	"	14.3	18.0	11.0
1912	Wild <i>G. morsitans</i>	500	"	13.9	18.0	9.0

COMPARISON OF THE CURVES FROM THE THREE STRAINS.

CHART 5.—Curves representing the Distribution, by Percentages, in respect to Length, of *T. pecorum*. 500 specimens from each of the Mvera Cattle Strain, the Wild-game Strain, and the Wild *Glossina morsitans* Strain.

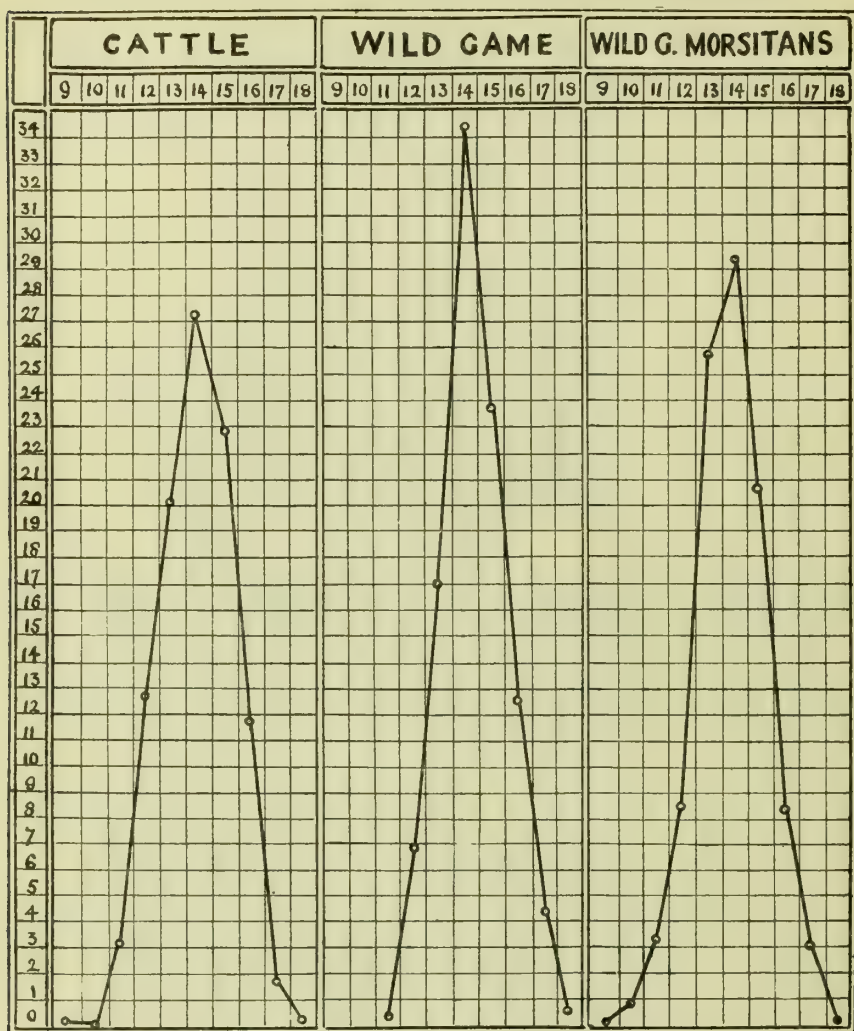


Table XII.—Distribution in respect to Length of 2,000 Individuals of the Three Strains of *T. pecorum*. The trypanosomes have been taken at random from various animals.

In microns.										
	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
Totals ...	2	6	42	193	452	618	453	178	51	5
Percentages ...	0.1	0.3	2.1	9.6	22.6	30.9	22.7	8.9	2.5	0.3

CHART 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 2,000 Individuals of the Three Strains of *T. pecorum*, Nyasaland, described in this paper.

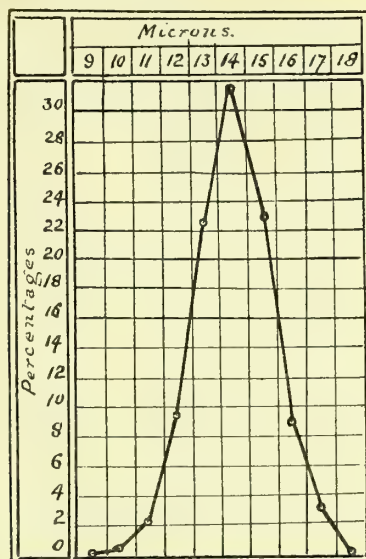


Table XIII.—Breadth of the Three Strains of *T. pecorum*. This does not include the undulating membrane.

Strain.	Average breadth.	Maximum breadth.	Minimum breadth.
Mvera cattle	1.97	3.0	1.25
Wild game	1.97	2.75	1.25
Wild <i>G. morsitans</i>	1.94	2.75	1.25
Average	1.96	2.83	1.25

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA PECORUM.

I. The Mvera Cattle Strain.

Table XIV.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Donkeys.					
1912.					
Jan. 26	291	Natural infection ...	?	—	Still alive after 377 days.
„ 26	292	„ ...	?	87 ?	Died of <i>T. pecorum</i> , April 22.
June 18	705	„ ...	?	—	Still alive after 233 days
„ 18	706	„ ...	?	—	„ „
„ 18	709	„ ...	?	—	„ „

* Duration includes the days of incubation ; it dates from day of infection.

Table XIV—continued.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912.					
Jan. 16	15a	Natural infection ...	?	—	Still alive after 387 days.
" 16	15b	" ...	?	—	" "
" 16	15c	" ...	?	—	" "
" 16	15d	" ...	?	?	Died " at Mvera of <i>T. pecorum</i> .
" 16	15e	" ...	?	?	" "
" 16	15f	" ...	?	?	" "
" 16	15g	" ...	?	?	" "
" 16	15h	" ...	?	?	" "
" 16	100	" ...	?	78 ?	Died of <i>T. pecorum</i> , April 28.
" 16	101	" ...	?	30 ?	Died of <i>T. pecorum</i> , Feb. 15.
" 16	102	" ...	?	—	Still alive after 387 days.
" 16	103	" ...	?	201 ?	Died of <i>T. pecorum</i> , Aug 28.
" 16	104	" ...	?	—	Still alive after 387 days.
" 16	105	" ...	?	—	" "
" 16	110	" ...	?	?	Died " at Mvera of <i>T. pecorum</i> .
" 16	174	" ...	?	?	" "
Feb. 16	226	From Dog 16 ...	11	—	Still alive after 356 days.
" 16	227	" 16 ...	7	—	" "
" 16	228	" 16 ...	11	—	" "
		Average ...	10	103	
Goats.					
Feb. 14	203	From Cow 103 ...	12	29	Died of <i>T. pecorum</i> .
" 14	205	" 103 ...	10	40	" "
" 14	206	" 103 ...	15	35	" "
" 14	208	" 103 ...	12	36	" "
" 16	178	From Dog 16 ...	12	38	" "
" 16	199	" 16 ...	11	209	" "
" 16	204	" 16 ...	10	—	Still alive after 356 days.
		Average ...	12	64	
Dogs.					
Jan. 17	16	From Cow 102 ...	26	30	Died of <i>T. pecorum</i> .
" 17	17	" 103 ...	13	56	" "
" 17	18	" 101 ...	—	—	Never showed trypanosomes.
" 17	19	" 100 ...	13	56	Died of <i>T. pecorum</i> .
Feb. 14	217	" 103 ...	16	28	" "
" 16	212	From Dog 16 ...	6	26	" "
" 16	214	" 16 ...	10	26	" "
" 16	218	" 16 ...	17	22	" "
" 16	225	" 16 ...	10	26	" "
		Average ...	14	34	

* Duration includes the days of incubation; it dates from day of infection.

Table XIV—continued.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Guinea-pigs.					
1912.					
Feb. 14	221	From Cow 103	...	—	Never showed trypanosomes.
" 14	222	" 103	...	—	" "
" 16	229	From Dog 16	...	—	" "
" 16	230	" 16	...	—	" "
Rats.					
Feb. 14	224	From Cow 103	...	—	Never showed trypanosomes.
" 16	231	From Dog 16	...	—	" "
" 16	232	" 16	...	—	" "
May 4	508	From Cow 103	...	—	" "
" 4	509	" 103	...	—	" "

Disease set up in Various Animals by Trypanosoma pecorum, Nyasaland—The Mvera Cattle Strain.

Donkeys.—When the Commission arrived at Kasu in January, 1912, the neighbouring Mvera Mission Station owned a herd of 16 donkeys. On examining them five were found to be infected with *T. pecorum*. One died after 87 days; the other four are at present free from trypanosomes and apparently in good health. It is true that three of the apparently recovered animals were given arsenic for some months, but the fourth, which got none, did equally well. It may also be noted that two of the four have recently given birth to healthy foals. Susceptible animals inoculated with the blood of the four donkeys remained healthy. From this it would appear that this strain of *T. pecorum* is not very fatal to donkeys.

Cattle.—A year ago the Mvera herd consisted of 50 head of cattle, and of these 16 were found to be infected with *T. pecorum*. At the present date 10 of these have died of this disease and six remain alive. The six have shown trypanosomes in their blood for some months, and their blood inoculated into healthy dogs has proved non-infective. In spite of this, however, these cattle remain in poor condition: thin, with rough, staring coats, and every appearance of suffering from a chronic disease, and it seems highly probable that more of them, if not all, will succumb to the effects of the trypanosome. Three oxen (Experiments 226, 227, and 228), which were inoculated from a dog whose blood contained *T. pecorum*, at the present date appear quite healthy and have apparently recovered.

Goats.—The Mvera Cattle strain is fatal to goats, killing them, as a rule, in 30 to 40 days. One goat lived 209 days, and another is still alive after a year and appears to have recovered.

* Duration includes the days of incubation; it dates from day of infection.

Monkeys.—No experiments were made on monkeys with the Mvera Cattle strain.

Dogs.—It is curious that this strain, which is harmless to guinea-pigs and white rats, is the most virulent of the three to dogs, killing them, without exception, in about 30 days.

Guinea-pigs and White Rats.—The Mvera Cattle strain has no effect on guinea-pigs or white rats. It is important to note how different strains of *T. pecorum* vary in their behaviour to the smaller laboratory animals.

II. The Wild-game Strain.

Table XV.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Goats.					
1912.					
Jan. 22	45	From Eland 44 ...	11	70	Died of <i>T. pecorum</i> .
Feb. 7	141	From Goat 45 ...	8	54	" "
Mar. 16	336	" 45 ...	9	48	" "
" 16	337	" 45 ...	9	20	" "
" 16	338	" 45 ...	9	41	" "
July 20	899	From Reedbuck 912	12	—	Still alive after 201 days.
" 23	898	From Hyæna 955 ...	13	89	Died of <i>T. pecorum</i> .
" 30	978	From Eland 1013 ...	13	93	" "
Aug. 11	1037	From Waterbuck 1061	8	23	Mixed infection.
" 18	1035	From Bushbuck 1078	18	—	Still alive after 172 days.
" 18	1037 ^a	" 1084	22	23	Died of <i>T. pecorum</i> .
" 19	1106	From Oribi 1096 ...	21	74	" "
" 24	1121	From Eland 1202 ...	16	82	" "
Sept. 4	1031	" 1044 ...	15	43	" "
" 7	1135	From Bushbuck 1261	19	85	" "
" 11	1307	From Buffalo 1304 ...	26	35	" "
" 17	1383	From Koodoo 1380 ...	6	68	" "
Oct. 6	1474	From Eland 1471 ...	22	—	Still alive after 123 days.
		Average ...	15	56	
Monkeys.					
April 9	403	From Dog 355 ...	9	236	Died of <i>T. pecorum</i> .
July 20	913	From Reedbuck 912	—	—	Never showed trypanosomes.
" 23	959	From Hyæna 955 ...	—	—	" "
" 30	1014	From Eland 1013 ...	27	101	Died of <i>T. pecorum</i> .
Aug. 11	1062	From Waterbuck 1061	—	—	Never showed trypanosomes.
" 18	1079	From Bushbuck 1078	—	—	" "
" 18	1085	" 1084	11	88	Died of <i>T. pecorum</i> .
" 19	1087	From Oribi 1096 ...	—	—	Never showed trypanosomes.
Sept. 7	1262	From Bushbuck 1261	—	—	" "
" 11	1305	From Buffalo 1304 ...	—	—	" "
" 17	1381	From Koodoo 1380 ...	—	—	" "
" 25	1478	From Hartebeeste 1453	—	—	" "
		Average ...	15	142	

* Duration includes the days of incubation; it dates from day of infection.

Table XV—continued.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dogs.					
1912.					
Jan. 22	46	From Eland 44 ...	—	—	Never showed trypanosomes.
Feb. 7	134	From Goat 45 ...	8	35	Died of <i>T. pecorum</i> .
" 7	138	" 45 ...	—	—	Never showed trypanosomes.
Mar. 20	355	" 45 ...	8	222	Died of <i>T. pecorum</i> .
" 20	356	" 45 ...	12	30	" "
" 20	357	" 45 ...	—	—	Never showed trypanosomes.
May 4	514	From Dog 355 ...	12	20	Died of <i>T. pecorum</i> .
" 4	515	" 355 ...	9	20	" "
July 20	914	From Reedbuck 912	—	—	Never showed trypanosomes.
" 23	963	From Hyæna 955 ...	—	—	" "
" 30	1015	From Eland 1013 ...	15	83	Died of <i>T. pecorum</i> .
Aug. 11	1063	From Waterbuck 1061	—	—	Never showed trypanosomes.
" 18	1080	From Bushbuck 1078	—	—	" "
" 18	1086	" 1084	8	156	Died of <i>T. pecorum</i> .
" 19	1098	From Oribi 1096 ...	—	—	Never showed trypanosomes.
" 27	1204	From Eland 1202 ...	27	—	Alive after 163 days.
Sept. 7	1263	From Bushbuck 1261	—	—	Never showed trypanosomes.
" 11	1306	From Buffalo 1304 ...	—	—	" "
" 17	1382	From Koodoo 1380 ...	—	—	" "
" 25	1455	From Hartebeeste 1453	8	24	Died of <i>T. pecorum</i> .
		Average ...	12	74	
Guinea-pig.					
Mar. 20	358	From Goat 45 ...	—	—	Never showed trypanosomes.
Rats.					
Mar. 20	360	From Goat 45 ...	—	—	Never showed trypanosomes.
" 21	361	" 45 ...	—	—	" "
May 4	510	From " Dog 355 ...	9	29	Died of <i>T. pecorum</i> .
" 4	511	" 355 ...	9	14	" "
Oct. 28	1518	" 355 ...	—	—	Never showed trypanosomes.
" 28	1519	" 355 ...	—	—	" "
Nov. 15	1518	From Monkey 403 ...	10	30	Died of <i>T. pecorum</i> .
" 15	1519	" 403 ...	17	30	" "
" 16	1608	From Goat 1135 ...	16	45	" "
		Average ...	12	30	

* Duration includes the days of incubation; it dates from day of infection.

*Disease set up in Various Animals by T. pecorum, Nyasaland—
The Wild-game Strain.*

Cattle.—No cattle were available for experiment.

Goats.—As might be expected, *T. pecorum* from the blood of antelope behaves in the same way to goats as that from cattle. It has the same virulence, gives rise to the same symptoms and *post-mortem* appearances.

Monkeys.—The monkey is an animal not very susceptible to *T. pecorum*. Blood taken from a wild animal and injected at the same time and under exactly the same conditions into a healthy goat, monkey, and dog, will often infect the first and last and fail to infect the monkey. In 10 experiments in which all the goats became infected, 8 of the 10 monkeys remained healthy. The disease is also much more chronic in the monkey than in the goat, the average duration of the disease in the former being 142 days, whereas in the latter it is only 56 days.

Dogs.—In comparison with the goat, the dog is also somewhat refractory to the Wild-game strain. In nine experiments all the dogs became infected, while their companion dogs, injected with the same blood, remained healthy.

Guinea-pigs.—The one guinea-pig inoculated remained unaffected.

White Rats.—This strain is irregular in its action towards white rats. In nine experiments four remained healthy, although the five which did become infected died, on an average, in 30 days, which is moderately rapid for *T. pecorum*.

III. *The Wild Glossina morsitans Strain.*

Table XVI.

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912. Feb. 3	108	Fly country ... ?	140		Mixed infection.
Goats.					
Jan. 25	89	Wild flies ...	15	60	Died of <i>T. pecorum</i> .
Feb. 3	125	" ...	7	36	Mixed infection.
" 8	175	" ...	20	88	" "
" 13	200	" ...	—	—	Never showed <i>T. pecorum</i> .
" 19	247	" ...	21	30	Mixed infection.
" 22	281	" ...	10	59	" "
Mar. 6	273	" ...	6	47	" "
" 9	270	" ...	—	—	Never showed <i>T. pecorum</i> .
" 18	343	" ...	11	34	Died of <i>T. pecorum</i> .
" 23	363	" ...	12	43	Mixed infection.
" 28	369	From Goat 109 ...	21	—	Still alive after 315 days
April 1	397	Wild flies ...	10	60	Mixed infection
" 3	408	" ...	11	40	Died of <i>T. pecorum</i> .

* Duration includes the days of incubation; it dates from day of infection.

Table XVI—continued.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Goats—continued.					
1912. April 10	410	Wild flies ...	—	—	Never showed <i>T. pecorum</i> .
" 15	414	" ...	10	36	Mixed infection.
" 18	428	" ...	—	—	Never showed <i>T. pecorum</i> .
" 19	422	" ...	7	69	Mixed infection.
" 23	420	" ...	—	—	Never showed <i>T. pecorum</i> .
" 24	415	" ...	16	49	Mixed infection.
" 29	429	" ...	12	43	" "
" 29	430	" ...	10	45	" "
May 4	434	" ...	9	—	Still alive after 277 days.
" 9	266	" ...	8	71	Mixed infection.
" 9	269	" ...	14	22	" "
" 15	416	" ...	5	19	" "
" 17	553	" ...	—	—	Never showed <i>T. pecorum</i> .
" 24	565	" ...	7	56	Mixed infection.
" 28	564	" ...	5	29	Died of <i>T. pecorum</i> .
" 28	568	" ...	9	24	Mixed infection.
" 31	570	" ...	4	64	" "
June 1	569	" ...	5	36	" "
" 1	571	" ...	11	23	" "
" 2	620	" ...	—	—	Never showed <i>T. pecorum</i> .
" 5	622	" ...	15 ?	34	Mixed infection.
" 8	627	" ...	12	24	" "
" 10	634	" ...	9	—	Still alive after 241 days.
" 10	635	" ...	13	63	Died of <i>T. pecorum</i> .
" 14	640	" ...	18	60	" "
" 17	645	" ...	16	32	" "
" 25	716	" ...	10	64	Mixed infection.
July 5	711	" ...	20	106	Died of <i>T. pecorum</i> .
" 9	795	" ...	10	40	" "
Oct. 14	1689	From Goat 1635 ...	9	38	Mixed infection.
" 28	1516	Wild flies ...	8	31	" "
" 31	1538	" ...	8	24	" "
Nov. 13	1588	" ...	9	—	Still alive after 85 days.
" 18	1613	" ...	—	—	Never showed <i>T. pecorum</i> .
" 27	1638	" ...	10	65	Mixed infection.
Dec. 5	1667	" ...	11	—	Still alive after 63 days.
" 6	1676	" ...	4	41	Mixed infection.
" 11	1685	" ...	6	38	" "
" 14	1693	From Goat 1634 ...	9	—	Still alive after 54 days.
" 19	1703	Wild flies ...	7	—	Still alive after 49 days.
Average ...			11	46	

* Duration includes the days of incubation; it dates from day of infection.

Table XVI—continued.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Pigs.					
1912.					
Nov. 18	1611	Wild flies ...	—	—	Never showed <i>T. pecorum</i> .
" 25	1636	" ...	13	—	Still alive after 73 days.
Dec. 3	1665	" ...	6	17	Mixed infection.
" 6	1674	" ...	3	10	" "
" 11	1683	" ...	—	—	Never showed <i>T. pecorum</i> .
" 16	1701	" ...	6	12	Mixed infection.
1913.					
Jan. 1	1725	" ...	6	21	Died of <i>T. pecorum</i> .
" 16	1771	From Dog 1702 ...	14	—	Still alive after 21 days.
" 21	1781	Wild flies ...	6	—	Still alive after 16 days.
		Average ...	8	15	
Monkeys.					
1912.					
Jan. 24	55	Wild flies ...	—	—	Never showed <i>T. pecorum</i> .
Feb. 8	58	" ...	—	—	Mixed " infection.
" 13	219	" ...	7	9	" "
" 20	49	" ...	—	—	Never showed <i>T. pecorum</i> .
April 10	404	" ...	—	—	" "
" 15	405	" ...	—	—	" "
" 18	447	" ...	—	—	" "
" 18	448	" ...	—	—	" "
" 19	450	From Dog 328 ...	24	202	Died of <i>T. pecorum</i> .
" 19	451	" 328 ...	17	165	" "
" 23	465	Wild flies ...	—	—	Never showed <i>T. pecorum</i> .
" 23	467	" ...	24	188	Died of <i>T. pecorum</i> .
" 26	488	" ...	—	—	Never showed <i>T. pecorum</i> .
" 27	495	" ...	—	—	" "
May 3	504	" ...	7	11	Mixed infection.
" 3	505	" ...	—	—	Never showed <i>T. pecorum</i> .
" 8	523	" ...	—	—	" "
" 9	521	" ...	—	—	" "
" 14	545	" ...	—	—	" "
" 29	596	" ...	34	150	Died of <i>T. pecorum</i> .
" 31	601	" ...	—	—	Never showed <i>T. pecorum</i> .
June 6	623	" ...	24	180	Died of <i>T. pecorum</i> .
" 7	625	" ...	—	—	Never showed <i>T. pecorum</i> .
" 11	629	" ...	—	—	" "
" 18	702	" ...	—	—	" "
" 25	739	" ...	—	—	" "

* Duration includes the days of incubation; it dates from day of infection.

Table XVI—continued.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Monkeys—continued.					
1912.					
July 3	788	Wild flies ...	—	—	Never showed <i>T. pecorum</i> .
Sept. 25	1452	" ...	21	34	Died of <i>T. pecorum</i> .
Oct. 29	1536	" ...	—	—	Never showed <i>T. pecorum</i> .
Nov. 11	1586	" ...	—	—	" "
		Average ...	20	117	
Dogs.					
Feb. 24	258	From Monkey 219	12	43	Died of <i>T. pecorum</i> .
" 28	256	From Goat 89	15	24	" "
Mar. 9	321	" 125	12	34	" "
" 9	322	" 125	9	48	" "
" 9	323	" 89	12	26	" "
" 9	324	" 89	12	24	" "
" 9	328	Wild flies ...	23	39	" "
" 17	341	" ...	15	28	" "
" 20	359	From Dog 258	8	14	" "
" 22	362	Wild flies ...	10	17	" "
April 3	400	" ...	16	18	" "
" 12	436	" ...	8	37	Mixed infection.
" 17	446	" ...	33	60	Died of <i>T. pecorum</i> .
" 20	463	" ...	21	57	" "
" 20	464	" ...	13	57	" "
" 24	478	" ...	18	56	" "
" 25	466	" ...	15	50	" "
" 25	468	" ...	11	21	" "
" 29	489	" ...	15	49	" "
" 30	496	" ...	12	46	" "
May 6	506	" ...	17	41	" "
" 6	507	" ...	19	42	" "
" 8	525	" ...	—	—	Never showed <i>T. pecorum</i> .
" 10	522	" ...	18	38	Died of <i>T. pecorum</i> .
" 17	549	From Monkey 523	10	51	Mixed infection.
" 17	551	Wild flies ...	8	25	" "
" 18	548	" ...	30	—	Still alive after 258 days.
" 24	578	" ...	18	24	Died of <i>T. pecorum</i> .
" 28	574	" ...	15	33	" "
" 29	595	" ...	3	22	Mixed infection.
" 29	599	" ...	22	152	Died of <i>T. pecorum</i> .
" 31	597	" ...	8	30	" "
" 31	602	" ...	5	—	Still " alive after 175 days.
June 2	619	" ...	10	28	Died of <i>T. pecorum</i> .
" 7	624	" ...	17	29	" "
" 8	626	" ...	8	54	Mixed infection.
" 9	630	" ...	13	21	Died of <i>T. pecorum</i> .
" 13	666	" ...	12	—	Still alive after 231 days.
" 19	701	" ...	11	19	Died of <i>T. pecorum</i> .
" 25	729	" ...	—	—	Never showed <i>T. pecorum</i> .

* Duration includes the days of incubation; it dates from day of infection.

Table XVI—continued.

Date.	No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dogs—continued.					
1912.					
July 4	734	Wild flies ...	14	—	Still alive after 210 days.
Oct. 29	1537	" ...	—	—	Never showed <i>T. pecorum</i> .
Nov. 12	1587	" ...	20	58	Died of <i>T. pecorum</i> .
" 19	1612	" ...	17	—	Still alive after 78 days.
" 19	1615	From Goat 1610 ...	9	17	Died of <i>T. pecorum</i> .
" 25	1637	Wild flies ...	—	—	Never showed <i>T. pecorum</i> .
Dec. 4	1666	" ...	12	32	Died of <i>T. pecorum</i> .
" 6	1675	" ...	—	—	Never showed <i>T. pecorum</i> .
" 11	1684	" ...	—	—	" "
" 14	1691	From Goat 1634 ...	9	21	Mixed infection.
" 14	1695	" 1635 ...	5	16	" "
" 18	1702	Wild flies ...	16	33	Died of <i>T. pecorum</i> .
		Average ...	14	37	
Guinea-pigs.					
April 19	452	From Dog 328 ...	13	42	Died of <i>T. pecorum</i> .
June 14	672	" 602 ...	—	—	Never showed <i>T. pecorum</i> .
" 14	673	" 602 ...	24	38	Died of <i>T. pecorum</i> .
" 14	674	" 547 ...	—	—	Never showed <i>T. pecorum</i> .
" 14	675	" 547 ...	24	44	Died of <i>T. pecorum</i> .
" 14	676	" 549 ...	—	—	Never showed <i>T. pecorum</i> .
" 14	677	" 549 ...	—	—	" "
" 14	680	" 551 ...	13	39	Mixed infection.
" 14	681	" 551 ...	20	42	" "
" 14	682	" 595 ...	—	—	Never showed <i>T. pecorum</i> .
" 14	683	" 595 ...	—	—	" "
		Average ...	19	41	
Rats.					
April 19	454	From Dog 328 ...	6	28	Died of <i>T. pecorum</i> .
" 19	455	" 328 ...	11	30	" "
June 11	657	" 551 ...	13	31	Mixed infection.
" 11	660	" 602 ...	5	70	" "
" 12	655	" 549 ...	—	—	Never showed <i>T. pecorum</i> .
" 14	658	" 595 ...	—	—	" "
Nov. 19	1616	" 1610 ...	—	—	" "
Dec. 14	1692	" 1634 ...	9	27	Mixed infection.
" 14	1696	" 1635 ...	—	—	Never showed <i>T. pecorum</i> .
		Average ...	9	37	

* Duration includes the days of incubation; it dates from day of infection.

*Disease set up in Various Animals by T. pecorum, Nyasaland—
The Wild G. morsitans Strain.*

Cattle.—Only one ox was experimented with. It was sent into the "fly-country," and as a result incurred a double infection of *T. pecorum* and *T. capræ*, and died in 140 days.

Goats.—The wild flies brought up from the "low-country" were fed, as a rule, on a goat, monkey, and dog. As the flies were often infected with several species of pathogenic trypanosomes, the result was many cases of mixed infection. In 50 experiments with goats, 10, or one-fifth only, were infections with *T. pecorum* alone. As *T. pecorum* is the most rapidly fatal trypanosome to goats among the various kinds of trypanosomes found in this locality, it may be assumed that the 28 goats which died of mixed infection owed this mainly to the *T. pecorum* infection.

Pig.—In eight feeding experiments with wild *G. morsitans*, only two showed a pure infection with *T. pecorum*. One died in 21 days and the other is still alive after 16 days. Another pig was inoculated from Dog 1702 and is still alive after 21 days. This species of domestic animal may, therefore, be said to be susceptible to this disease.

Monkey.—To this strain as to the Wild-game strain the monkey shows its refractoriness. These animals had the same chance of taking the disease as the goats and the dogs, but in 28 experiments the monkeys only became infected six times (21.4 per cent.), whereas in 50 goats there were 42 infections (84 per cent.), and in 41 dogs 35 (85.4 per cent.). The duration of the disease in the monkey is also long, the average in four cases being 138 days.

Dogs.—The Wild *G. morsitans* strain is fatal to dogs, killing them, on an average, in 41 days. In 35 cases of this disease among dogs, only five have survived to the present date. One of these may be said to have recovered, as the trypanosomes disappeared from the blood and the blood ceased to be infective. The other four still show trypanosomes in the blood after 258, 231, 210, and 78 days respectively. The dog may, therefore, be said to be as susceptible to the Wild *G. morsitans* strain of *T. pecorum* as the goat, few escaping the disease when bitten by an infective fly.

Guinea-pigs and White Rats.—The susceptibility of these animals to the Wild *G. morsitans* strain would be considered by some to go to prove that exaltation of virulence obtains after passage through the fly. This, however, is doubtful. Passage through the fly would more probably restore the trypanosome to its normal form—sometimes increasing, sometimes decreasing, its virulence for a particular animal.

COMPARISON OF THE THREE STRAINS IN REGARD TO THEIR
VIRULENCE TOWARDS VARIOUS ANIMALS.

Table XVII.—The Average Duration of the Disease in Various Animals, in days. The letter R means that the animal is refractory, that is, not susceptible to the disease.

Strain.	Donkey.	Ox.	Goat.	Pig.	Monkey.	Dog.	Guinea-pig.	White rat.
Mvera Cattle ...	87 ?	103 ?	64			34	R	R
Wild Game ...			56		142	74	R	30
Wild <i>G. morsitans</i>		140	46	21	117	37	41	37

On the whole it may be concluded that the Wild *G. morsitans* strain is the most virulent.

Table XVIII.—The Percentages of Recoveries in Various Animals from the Three Strains. The letter R stands for refractory.

Strain.	Donkey.	Ox.	Goat.	Pig.	Monkey.	Dog.	Guinea-pig.	White rat.
Mvera Cattle ...	80	37	14			0	R	R
Wild Game ...			17		0	0	R	0
Wild <i>G. morsitans</i>		0	6		0	2	0	0

It must be confessed that the margin of error in a table such as this must be enormous; the fallacies are many. It is difficult or impossible to say when or if an animal has recovered from a trypanosome disease. The numbers employed are often small. For example, there is 0 per cent. indicated in regard to Guinea-pig, Wild-game strain. This animal is returned as refractory, but there was only one experiment. It is evident that it would be more satisfactory if, say, a minimum of 10 animals of each species was used in studying each different strain. But in the wilds of Nyasaland this is a counsel of perfection impossible to attain. At the present time the Commission finds the greatest difficulty in obtaining small numbers of such local animals as goats, monkeys, and dogs, while as to the smaller laboratory animals, they must come from England. This entails a heavy mortality, as may be judged from the fact that of the last consignment of 25 guinea-pigs and 50 rats only 9 of the former and 12 of the latter arrived at Kasu alive.

THE AVERAGE DURATION OF LIFE, IN DAYS, OF VARIOUS ANIMALS
INFECTED BY *T. PECORUM*, NYASALAND.

The following table combines the results obtained from a study of the susceptibility of various animals to each of the three strains, so as to arrive at a numerical expression of the virulence

of the *T. pecorum* of Nyasaland. It must be carefully noted that only fatal cases are taken into account. In human medicine duration of disease means the number of days between the beginning of the illness and recovery. Here it means the number of days between infection and death. The table also shows against which animal the energies of *T. pecorum* are chiefly directed. It is certain that it is only by the detailed study of many strains of each of the African species of trypanosomes that a satisfactory classification of this important group of hæmatozoa will be arrived at. This work must be done on the spot, where the natural conditions for the normal life of the parasite are present, and where frequent opportunity of passing through the invertebrate host—the tsetse fly—exists. It is absurd to expect to arrive at any classification at all approaching a true one by the study of strains of trypanosomes kept for many years and undergoing many vicissitudes in our European laboratories.

Table XIX.—The Average Duration of Life, in days, of Various Animals infected by *T. pecorum*.

—	Donkey.	Cattle.	Goat.	Pig.	Monkey.	Dog.	Guinea-pig.	White rat.
Average duration, in days.	87 ?	121 ?	55	21	129	48	41	33
Number of animals employed.	1	4	59	1	11	57	5	19

Table XX.—The Percentages of Recoveries in Various Animals from *T. pecorum* Infection.

—	Donkey.	Cattle.	Goat.	Pig.	Monkey.	Dog.	Guinea-pig.	White rat.
Percentages ...	80	35	12	0	0	1	0	0
Number of animals employed.	5	17	70	1	11	63	5	10

THE CARRIER OF *T. PECORUM*, NYASALAND.

In Nyasaland the carrier is *G. morsitans*, of which 4·6 per 1,000 were found infected by this species. This is the minimum, but the real proportion of infected flies is probably three or four times as great. An account of the development of *T. pecorum* in this tsetse fly will be dealt with in a future paper. There is also some evidence derived from the outbreak among the Mvera cattle that, given infected animals in a herd, it is possible that

Tabanidæ Hæmatopota, or other biting flies may act as mechanical carriers. The evidence that *Stomoxys* plays a similar rôle is unsatisfactory.

Conclusions.

1. The Mvera Cattle strain, the Wild-game strain and the Wild *G. morsitans* strain belong to the same species of trypanosome, *T. pecorum*.

2. *T. pecorum*, Nyasaland, is identical with the species found and described in Uganda.

3. It is an important disease of domestic animals in Nyasaland, being destructive to donkeys, oxen, goats, pigs, and dogs.

4. Its carrier in this district is *G. morsitans*, about 2 per cent. probably of the local wild flies being naturally infected with *T. pecorum*.

5. Its reservoir is the wild game inhabiting "fly-country," 14.4 per cent. of which were found to be infected with this trypanosome. It is hardly to be doubted that 100 per cent. are, or have been, infected.

6. It is recommended that if infected animals are found in a herd they should be destroyed or segregated, as there is a danger of biting flies other than the tsetse spreading the disease in the herd by mechanical transmission.

X.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND.—THE MZIMBA STRAIN.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; and Lady BRUCE, R.R.C.

(Received May 5, 1913.)

[Plates 5-7.]

INTRODUCTION.

Up to the present time it has usually been considered that almost all the cases of Human trypanosome disease in man in Nyasaland have been confined to a small area. This, the so-called Sleeping-Sickness District, has been described in a former paper,[†] but it may be repeated here that it is the part of the "fly-country" lying along the western shore of Lake Nyasa between S. Lat. 13° 20' and 13° 50', and extending some twenty miles inland. Through the centre of this area a road runs from Domira Bay on the Lake into North-East Rhodesia. This road, until lately, was a principal highway between the coast and Central Africa. Dr. Aylmer May, the Principal Medical Officer of North-East Rhodesia, who lately visited Kasu, informed the Commission that it was along this trade-route that all the North-East Rhodesian cases of Human trypanosome disease have occurred. It is said that some 25,000 native porters passed along this road every year, and as they entered a *Glossina palpalis* area at the Congo end of their journey, it seemed at first natural to suspect that the disease was true Sleeping Sickness, and had spread from west to east along this trade-route. This suspicion was shown to be groundless by the discovery that the parasite causing the disease in North-East Rhodesia and Nyasaland is not *Trypanosoma gambiense*, but a distinct and separate species giving rise to a totally different disease. The question then arose as to whether this was an imported or indigenous disease. It has, therefore, been one of the objects of this Commission to determine whether the trypanosome causing Human trypanosome disease in Nyasaland is restricted to the game and "fly" of the Proclaimed Area, or if it extends to the north and south along the "fly-belt." If it is found to extend over all the "fly-area" in Nyasaland, then the disease is probably native to the soil and not an importation from Tanganyika or the Congo.

But it will be well at this point to lay down definitely the various opinions or theories at issue. These are three in number.

First, that the Human trypanosome disease of North-East Rhodesia and Nyasaland is caused by a specific trypanosome, *T. rhodesiense*, that the wild game and "fly" are heavily infected with it, and that *T. brucei*, or Nagana, is absent altogether. This is the theory held by one school.

* Reprinted from the Proceedings of the Royal Society, B, vol. 87.

† 'Roy. Soc. Proc.,' B, vol. 86, p. 274. (*Supra*, p. 20.)

Second, that the wild game and "fly" are heavily infected throughout these "fly-areas" by *T. brucei*, but that at certain places or foci another trypanosome, *T. rhodesiense*, occurs, which is pathogenic to man as well as the other animals. That these two species of trypanosomes are indistinguishable morphologically or by their action on animals, except that one is capable of infecting man and the other not. That the only way to separate them is by inoculating man: if the man reacts it is *T. rhodesiense*, if not, *T. brucei*.

Third, that *T. brucei*—a common trypanosome of wild game, whose distribution extends from Zululand to the Sudan—and *T. rhodesiense* are one and the same species of trypanosome, and that wherever wild game and *G. morsitans* are found there also will be found cases of trypanosome disease in man. That the cause of the sparsity of cases in man in these areas is due to the fact that man is more or less refractory to the trypanosome, and that it is only rarely that the "fly" meets with a susceptible subject. That this is the reason why the cases of Human trypanosome disease in the Luangwa valley and in Nyasaland do not tend to increase in numbers. The disease remains stationary, as it probably has done during the last thousand years. This is the working hypothesis held at present by this Commission.

These, then, are the points at issue, and it would appear at present that the only way of solving the problem will be by searching and finding out whether cases of *T. brucei* disease, or Nagana, in man occur wherever *G. morsitans* and this parasite are found together. Already cases have been found on the Rovuma river on the borders of German and Portuguese East Africa, and in the Hartley District south of the Zambesi, in Europeans and natives, who certainly could only have contracted the disease in these widely separated districts.

Thanks to Mr. Garden, the Government Veterinary Officer, the Commission have had the opportunity of studying a trypanosome of the Nagana type found in the blood of a donkey at Mzimba (lat. $11^{\circ} 55' S.$, long. $33^{\circ} 35' E.$), about 100 miles north of the northern border of the Proclaimed Area. It is proposed, then, in this paper to describe this trypanosome, in pursuance of the policy of describing in detail as many strains as possible of this type of trypanosome, if peradventure some method of separating *T. brucei* from *T. rhodesiense* may be discovered, or of proving them to be one and the same species.

MORPHOLOGY OF THE MZIMBA STRAIN.

A. *Living, Unstained.*

The movements of this trypanosome in the living condition are similar to *T. brucei* and *T. gambiense* in being non-translatory.

B. *Fixed and Stained.*

The blood films were fixed, stained and measured as previously described in the "Proceedings."*

* 'Roy. Soc. Proc.' B, vol. 81, pp. 16 and 17.

Table I.—Measurements of the Length of the Trypanosome of the Mzimba Strain.

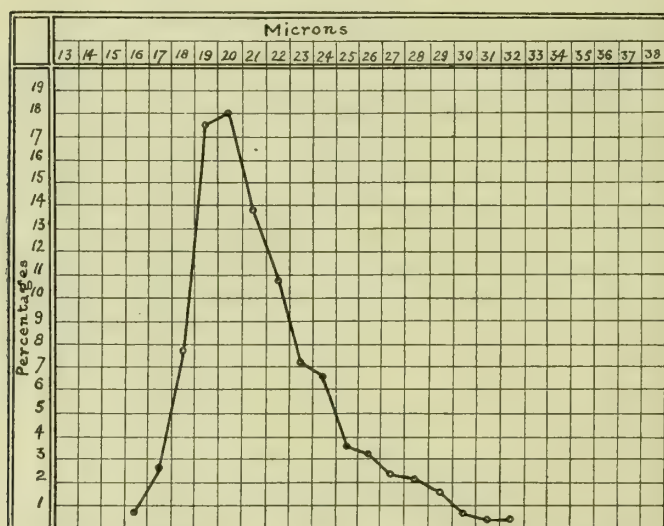
Date.	Number of experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Mar. 28	365	Donkey	Osmic acid	Giemsa	18.3	21.0	16.0
" 2	384	Goat ...	"	"	19.8	26.0	18.0
" 27	368	" ...	"	"	20.1	23.0	17.0
Apr. 22	381	" ...	"	"	20.2	25.0	18.0
" 29	384	" ...	"	"	22.8	32.0	18.0
" 29	402	Monkey	"	"	21.2	30.0	17.0
May 6	402	" ...	"	"	21.6	29.0	18.0
Mar. 27	366	Dog ...	"	"	22.7	30.0	19.0
" 27	367	" ...	"	"	22.2	29.0	19.0
" 28	366	" ...	"	"	22.0	30.0	19.0
" 28	367	" ...	"	"	21.6	30.0	19.0
" 29	366	" ...	"	"	22.0	32.0	17.0
" 29	367	" ...	"	"	20.9	24.0	19.0
" 30	366	" ...	"	"	22.3	29.0	17.0
Apr. 1	366	" ...	"	"	20.2	24.0	17.0
" 4	366	" ...	"	"	20.9	25.0	16.0
" 4	366	" ...	"	"	21.6	26.0	17.0
" 8	366	" ...	"	"	21.8	31.0	19.0
" 8	366	" ...	"	"	20.3	27.0	17.0
" 8	387	" ...	"	"	23.1	29.0	17.0
" 8	388	" ...	"	"	19.6	23.0	18.0
" 11	366	" ...	"	"	22.0	31.0	18.0
" 11	388	" ...	"	"	19.6	21.0	18.0
" 15	387	" ...	"	"	22.0	30.0	18.0
" 15	388	" ...	"	"	20.6	25.0	16.0
May 9	512	Rat	"	"	23.3	31.0	19.0
" 9	512	" ...	"	"	24.3	29.0	19.0
" 9	513	" ...	"	"	22.6	28.0	16.0
" 9	513	" ...	"	"	21.8	26.0	16.0
" 10	512	" ...	"	"	21.2	26.0	19.0
" 10	512	" ...	"	"	21.1	27.0	18.0
" 10	513	" ...	"	"	20.9	25.0	18.0
" 10	513	" ...	"	"	20.3	23.0	17.0
" 11	512	" ...	"	"	22.1	28.0	18.0
" 11	512	" ...	"	"	21.0	27.0	18.0
" 11	513	" ...	"	"	20.2	24.0	17.0
" 11	513	" ...	"	"	22.2	30.0	18.0
" 13	512	" ...	"	"	20.4	25.0	18.0
" 13	512	" ...	"	"	20.2	26.0	18.0
" 13	513	" ...	"	"	19.4	26.0	17.0
" 13	513	" ...	"	"	19.3	23.0	17.0
" 14	512	" ...	"	"	21.7	28.0	17.0
" 14	512	" ...	"	"	21.5	28.0	17.0
" 14	513	" ...	"	"	21.7	28.0	19.0
" 14	513	" ...	"	"	22.5	29.0	19.0
" 15	512	" ...	"	"	24.1	30.0	17.0
" 15	512	" ...	"	"	24.5	32.0	18.0
" 15	513	" ...	"	"	21.2	26.0	19.0
" 15	513	" ...	"	"	21.6	28.0	18.0
" 16	513	" ...	"	"	21.2	31.0	18.0
					21.4	32.0	16.0

The average length of the trypanosome of the Mzimba strain, in different species of animals, taken from Table I., is as follows:—

Table II.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Donkey	20	18·3	21·0	16·0
Goat	80	20·7	32·0	17·0
Monkey	40	21·4	30·0	17·0
Dog	360	21·4	32·0	16·0
Rat	500	21·6	32·0	16·0

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,000 Individuals of the Trypanosome of the Mzimba Strain.

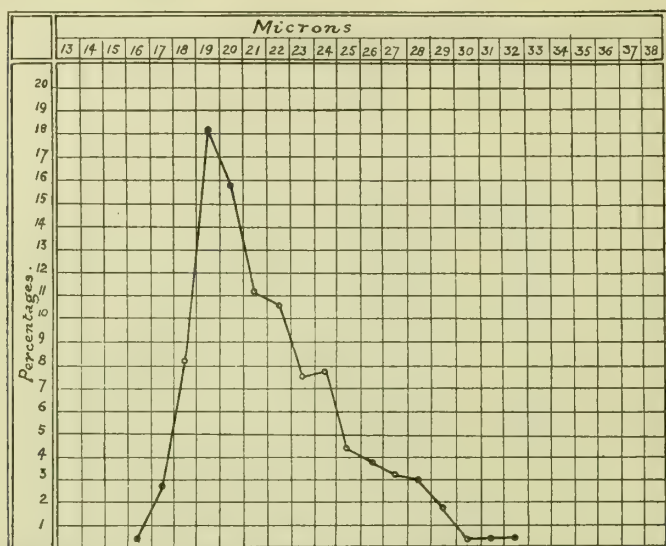


This curve is made up of measurements from 20 specimens of trypanosomes taken from the original donkey, 80 from the goat, 40 from the monkey, 360 from the dog, and 500 from the rat.

Table III.—Distribution in respect to Length of 1,000 Individuals of the Trypanosome of the Mzimba Strain.

Animal.	In microns.																	Average length.
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	
Donkey ...	3	1	6	7	2	1	—	—	—	—	—	—	—	—	—	—	—	18.3
Goat ...	—	—	5	6	4	2	1	1	—	—	1	—	—	—	—	—	—	19.8
" ...	—	1	—	6	8	3	2	1	—	—	—	—	—	—	—	—	—	20.1
" ...	—	—	4	5	4	2	2	1	1	1	—	—	—	—	—	—	—	20.2
" ...	—	—	1	2	4	2	2	2	—	3	—	—	1	1	—	—	1	22.8
Monkey ...	—	3	4	1	4	2	—	1	1	—	—	1	1	1	1	—	—	21.2
" ...	—	—	1	2	6	4	2	1	2	—	—	—	1	1	1	—	—	21.6
Dog ...	—	—	—	1	3	6	4	1	1	1	—	1	1	1	1	—	—	22.7
" ...	—	—	—	1	1	9	3	2	1	1	—	1	—	1	—	—	—	22.2
" ...	—	—	—	1	4	5	3	5	—	1	—	—	—	—	1	—	—	22.0
" ...	—	—	—	5	1	5	4	2	2	—	—	—	—	—	—	1	—	21.6
" ...	—	—	—	1	5	6	2	1	—	—	2	1	—	—	—	—	1	22.0
" ...	—	1	—	3	3	8	5	—	1	—	—	—	—	—	—	—	—	20.9
" ...	—	—	—	2	3	2	2	2	5	1	1	—	—	—	—	—	—	22.3
" ...	—	2	—	6	4	3	3	1	1	—	—	—	—	1	—	—	—	20.2
" ...	2	1	—	3	1	4	4	2	1	2	—	—	—	—	—	—	—	20.9
" ...	—	1	2	1	4	2	4	—	2	2	1	3	—	—	—	—	—	21.6
" ...	—	—	—	5	4	4	2	2	—	—	2	—	—	—	—	1	—	21.8
" ...	—	1	4	6	3	1	2	—	—	1	—	2	—	—	—	—	—	20.3
" ...	—	1	—	2	1	3	2	2	2	1	3	1	1	1	—	—	—	23.1
" ...	—	—	4	6	7	1	—	2	—	—	—	—	—	—	—	—	—	19.6
" ...	—	—	2	5	2	2	2	1	1	1	1	1	1	—	—	1	—	22.0
" ...	—	—	3	4	11	2	—	—	—	—	—	—	—	—	—	—	—	19.6
" ...	—	—	1	1	6	1	4	4	1	—	—	—	1	—	1	—	—	22.0
" ...	1	—	1	3	6	3	3	2	2	1	—	—	—	—	—	—	—	20.6
Rat ...	—	—	—	2	1	5	—	1	1	2	4	2	—	1	—	—	1	23.3
" ...	—	—	—	3	1	—	2	2	2	1	3	3	—	3	—	—	—	24.3
" ...	1	1	1	2	2	2	3	1	5	—	2	2	1	—	—	—	—	22.6
" ...	1	2	1	1	2	2	3	—	1	5	2	—	—	—	—	—	—	21.8
" ...	—	—	—	4	5	2	6	—	2	2	1	—	—	—	—	—	—	21.2
" ...	—	—	1	4	6	3	1	1	2	1	—	1	—	—	—	—	—	21.1
" ...	—	—	2	1	7	3	3	2	1	1	—	—	—	—	—	—	—	20.9
" ...	—	—	1	1	8	2	1	3	4	—	—	—	—	—	—	—	—	20.3
" ...	—	1	1	6	1	3	1	1	3	2	—	1	1	—	—	—	—	22.1
" ...	—	—	2	2	4	7	—	3	1	—	—	1	—	—	—	—	—	21.0
" ...	—	2	3	3	5	1	2	1	3	—	—	—	—	—	—	—	—	20.2
" ...	—	—	2	4	—	6	1	1	1	1	1	—	2	1	—	1	—	22.2
" ...	—	—	3	5	5	1	2	2	1	1	—	—	—	—	—	—	—	20.4
" ...	—	—	2	9	2	3	2	—	1	1	—	1	—	—	—	—	—	20.2
" ...	—	2	3	9	3	1	1	—	—	—	1	—	—	—	—	—	—	19.4
" ...	—	1	8	4	2	2	1	2	—	—	—	—	—	—	—	—	—	19.3
" ...	—	1	1	3	2	2	3	3	3	1	—	—	1	—	—	—	—	21.7
" ...	—	3	2	3	2	1	2	1	2	—	—	2	2	—	—	—	—	21.5
" ...	—	—	—	6	4	—	4	1	2	—	1	1	1	—	—	—	—	21.7
" ...	—	—	—	4	2	—	4	3	1	—	1	—	1	2	—	—	—	22.5
" ...	—	1	2	1	1	—	2	1	2	2	1	1	3	2	1	—	—	24.1
" ...	—	—	2	1	1	1	3	1	2	1	2	1	2	1	2	—	2	24.5
" ...	—	—	—	7	1	5	1	3	1	—	2	—	—	—	—	—	—	21.2
" ...	—	—	1	2	8	3	2	—	1	1	1	—	—	1	—	—	—	21.6
" ...	—	—	2	4	5	4	—	2	1	—	—	—	1	—	—	1	—	21.2
Total ...	8	27	79	175	180	139	109	72	66	36	32	24	22	16	7	4	4	
Percentages	0.8	2.7	7.9	17.5	18.0	13.9	10.9	7.2	6.6	3.6	3.2	2.4	2.2	1.6	0.7	0.4	0.4	

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of the Mzimba Strain, taken from Rats alone.



The similarity in the curve of the Mzimba strain and the curve of the Wild-game strain* is remarkable, and there can be little doubt that the same trypanosome is being dealt with. This is what might be expected, seeing there is probably only one animal between the wild *G. morsitans* and the experimental animal in both cases.

Table IV.—Measurements giving the Average Distance from the Posterior Extremity to Micronucleus, Micronucleus to Nucleus, etc., of 1,000 Individuals of the Trypanosome of the Mzimba Strain.

Posterior extremity to micronucleus.	Micronucleus to nucleus.	Diameter of nucleus.†	Nucleus to anterior extremity.	Flagellum.
Short and Stumpy (16–21 microns), 608 Individuals.				
1.2	4.4	2.9	10.6	0.4
Intermediate (22–24 microns), 247 Individuals.				
1.5	5.3	3.0	10.4	2.6
Long and Slender (25–32 microns), 145 Individuals.				
1.8	5.8	3.0	10.9	5.5
Average 1.5	5.2	3.0	10.6	2.8

* 'Roy. Soc. Proc.' B, vol. 86, p. 394.

† These measurements are made along the long axis of the trypanosome, and therefore if an oval nucleus is lying transversely, the measurement given will not represent the greatest length of the nucleus.

Table IV. agrees very closely with the same table given in the paper on the "Morphology of the Trypanosome causing Disease in Man in Nyasaland."* It is doubtful if this detailed method of measurement assists in putting trypanosomes in their proper places, but it may be that when more work has been done, something may emerge. There can be little doubt that morphology, in future, will play an important part—perhaps the most important part—in the classification of trypanosomes.

Breadth.—The average breadth is 3.45 microns (maximum 5.75, minimum 1.5). The short and stumpy average 3.7 (maximum 5.75, minimum 2), the intermediate 3.16 (maximum 5, minimum 1.5), and the long and slender 2.84 (maximum 4.25, minimum 1.75). The breadth of the widest part of the body of the 1,000 trypanosomes is measured, including the undulating membrane.

Shape.—This trypanosome closely resembles in general appearance the various strains which have already been described from man, wild game and wild *G. morsitans*. Three black-and-white plates are given (Plates 5-7), which will give a better idea of the appearance of this strain than a written description.

Table V.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Mzimba Strain.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.	Proportion to all forms per 1,000.
1912.				
Mar. 27 ...	367	Dog	0	0
" 28 ...	367	"	10	60
" 28 ...	366	"	3	18
" 29 ...	367	"	8	48
" 29 ...	366	"	13	78
" 30 ...	366	"	12	72
Apr. 1 ...	366	"	7	42
" 25 ...	387	"	15	90
" 29 ...	387	"	25	150
May 2 ...	387	"	37	222
" 10 ...	513	Rat	11	66
" 10 ...	512	"	22	132
" 11 ...	513	"	19	114
" 11 ...	512	"	12	72
" 13 ...	513	"	7	42
" 13 ...	512	"	5	30
" 14 ...	513	"	10	60
" 15 ...	513	"	28	168
" 16 ...	513	"	33	198
" 16 ...	512	"	3	18
Average			14.7	88

* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 428.

Micronucleus.—Situating, on an average, 1·8 microns from the posterior extremity in the long and slender, 1·5 in the intermediate, and 1·2 in the short and stumpy.

Undulating Membrane.—Well developed.

Flagellum.—The flagellum in the short and stumpy averages 0·4 micron (maximum 4, minimum 0·2), in the intermediate 2·6 (maximum 6, minimum 1) and in the long and slender 5·5 (maximum 10, minimum 2). Total average 2·8 (maximum 10, minimum 1).

Table VI.—Number of Flagellated and Non-flagellated Forms found among 1,000 Trypanosomes of the Mzimba Strain.

Short and stumpy.			Intermediate.			Long and slender.		
Length, microns.	Non-Flagellated.	Flagellated.	Length, microns.	Non-Flagellated.	Flagellated.	Length, microns.	Non-Flagellated.	Flagellated.
16	8	0	22	34	75	25	0	36
17	27	0	23	12	60	26	0	32
18	73	6	24	5	61	27	0	24
19	154	21				28	0	22
20	153	27				29	0	16
21	94	45				30	0	7
						31	0	4
						32	0	4
Totals	509	99		51	196		0	145

If the trypanosomes are divided into non-flagellar and flagellar, there are 56 per cent. of the former and 44 per cent. of the latter.

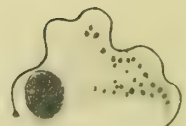
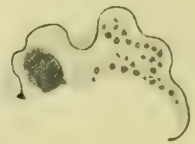
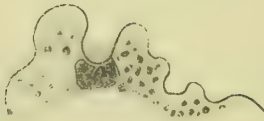
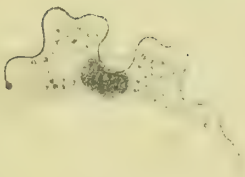
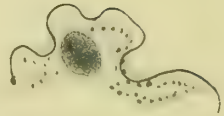
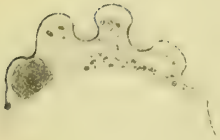
If we divide the 1,000 Mzimba trypanosomes by length into short and stumpy (16 to 21 microns), intermediate (22 to 24 microns), and long and slender (25 to 32 microns), the percentages are 60·8, 24·7, and 14·4 respectively.

Conclusions.

1. The trypanosome of the Mzimba strain is the same species as that occurring in the wild game inhabiting the Proclaimed Area, Nyasaland.

2. It has already been concluded that this species is *T. brucei* vel *rhodesiense*.

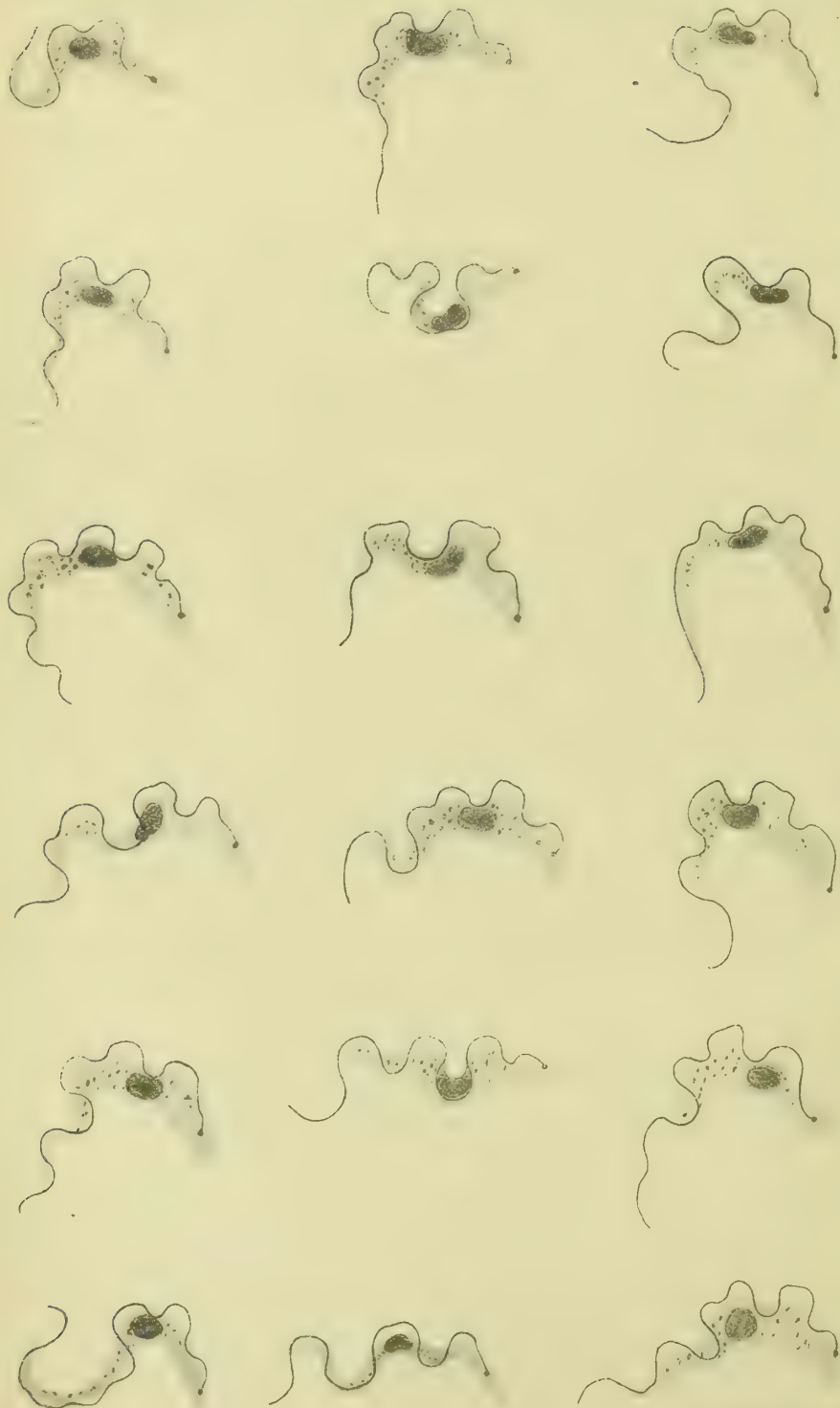
3. Hence it would appear that wild *G. morsitans* occurring in a district 100 miles north of the Proclaimed Area are infected with the trypanosome which causes the Human trypanosome disease of Nyasaland.



M. E. Bruce, del.

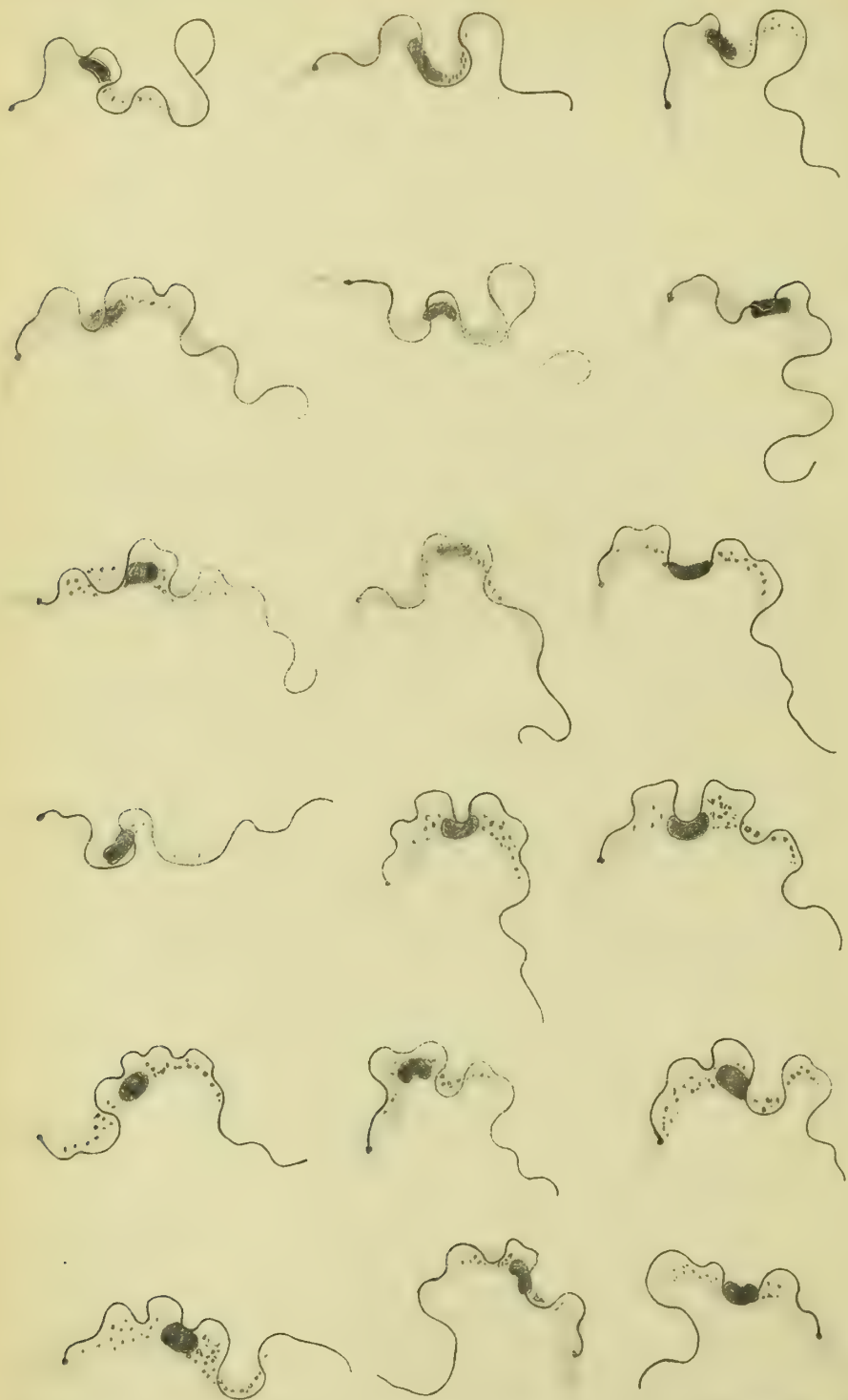
Huth, lith. London.

Short & Stumpy



M. E. Bruce del.

Huth Lith^r London.



M. E. Bruce, del.

Long & Slender

Hith Litho London.

DESCRIPTION OF PLATES.

Plate 5.

T. brucei vel *rhodesiense*.—Short and stumpy forms, 16-21 microns in length. Stained Giemsa. $\times 2,000$.

Plate 6.

T. brucei vel *rhodesiense*.—Intermediate forms, 22-24 microns in length. Stained Giemsa. $\times 2,000$.

Plate 7.

T. brucei vel *rhodesiense*.—Long and slender forms, 25-32 microns in length. Stained Giemsa. $\times 2,000$.

XI.—THE TRYPANOSOME CAUSING DISEASE IN MAN
IN NYASALAND.—SUSCEPTIBILITY OF ANIMALS
TO THE HUMAN STRAIN.

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; and Lady BRUCE, R.R.C.

(Received May 26, 1913.)

INTRODUCTION.

In previous papers the morphology of various strains of this trypanosome—from man, wild game and wild *Glossina morsitans*—was described, and the different strains compared.

This paper describes the action on animals of the five strains derived from cases of trypanosome disease in man in Nyasaland, and compares their virulence. In a future paper it is proposed to describe in the same way the action on animals of the Wild-game and Wild *G. morsitans* strains, but up to the present this has been impossible on account of the scarcity of experimental animals.

* Reprinted from the Proceedings of the Royal Society, B, Vol. 87.

ANIMALS SUSCEPTIBLE TO THE TRYPANOSOME CAUSING DISEASE
IN MAN IN NYASALAND. I.—THE HUMAN STRAIN.

I. *Strain I., Mkanyanga.*

Table I.

Date.	Num-ber of experi-ment.	Source of virus.	Period of incu-bation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912.					
Feb. 7	167	From Rat 36 ...	—	—	Never showed trypanosomes.
" 7	168	" 36 ...	13	134	Died of Strain I.
April 23	473	From Guinea-pig 373	—	—	Never showed trypanosomes.
" 23	474	" 373	9	—	Still alive after 325 days.
Goats.					
Feb. 7	142	From Rat 36 ...	8	33	Died of Strain I.
" 7	144	" 36 ...	8	19	" "
" 7	146	" 36 ...	—	—	Never showed trypanosomes.
" 7	149	" 36 ...	22	27	Died of Strain I
April 23	411	From Guinea-pig 373	9	55	" "
" 23	417	" 373	6	25	" "
" 23	418	" 373	44	45	" "
		Average ...	16.2	34.0	
Sheep.					
Feb. 7	162	From Rat 36 ...	2	19	Died of Strain I.
" 7	163	" 36 ...	2	23	" "
" 7	164	" 36 ...	2	33	" "
April 23	475	From Guinea-pig 373	9	16	" "
" 23	476	" 373	9	53	" "
" 23	477	" 373	13	57	" "
		Average ...	6.2	33.5	
Baboon.					
1911.					
Dec. 21	12	From Monkey 3 ...	—	—	Never showed trypanosomes.
Monkeys.					
Nov. 16	3	Mkanyanga ...	14 ?	35	Died of Strain I.
1912.					
Jan. 19	24	From Guinea-pig 13 ...	11 ?	28	" "
Feb. 16	233	From Monkey 24 ...	3	28	" "
" 16	234	" 24 ...	3	16	" "
April 23	469	From Guinea-pig 373	13	31	" "
" 23	470	" 373	6	42	" "
		Average ...	6.2	30.0	

* Duration includes the days of incubation; it dates from day of infection.

Table I.—*continued.*

Date.	Num-ber of experi-ment.	Source of virus.	Period of incu-bation, in days.	Duration of disease, in days.*	Remarks.
Dogs.					
1911.					
Oct. 15	1	Mkanyanga ...	11 ?	46	Died of Strain I.
Nov. 16	2	From Dog 1 ...	14 ?	21	" "
Dec. 7	7	" 2 ...	8	20	" "
" 20	11	" 7 ...	9	30	" "
1912.					
Jan. 15	14	" 11 ...	8	14	" "
" 31	111	" 14 ...	6	14	" "
Average ...			7·7	24·2	
Rabbit.					
June 14	671	From Sheep 476 ...	12	42	Died of Strain I.
Guinea-pigs.					
1911.					
Dec. 16	5	From Rat 4 ...	13 ?	13	Died of Strain I.
" 21	13	From Monkey 3 ...	11	100	" "
1912.					
Feb. 7	165	From Rat 36 ...	12	42	" "
" 7	166	" 36 ...	15	36	" "
Mar. 27	372	From Guinea-pig 13 ...	19	72	" "
" 27	373	" 13 ...	15	—	Killed April 23.
Average ...			15·2	52·5	
Rats.					
1911.					
Nov. 28	4	From Dog 1 ...	8	19	Died of Strain I.
Dec. 16	8	From Rat 4 ...	—	—	Never showed trypanosomes.
" 16	9	" 4 ...	?	13	Died of Strain I.
" 29	36	" 9 ...	25 ?	—	Killed.
" 29	27	" 9 ...	24 ?	26	Died of Strain I.
" 29	38	From Monkey 3 ...	25 ?	33	" "
1912.					
Feb. 16	235	" 24 ...	4	25	" "
" 16	236	" 24 ...	4	14	" "
April 23	471	From Guinea-pig 373...	7	15	" "
" 23	472	" 373...	6	22	" "
Nov. 8	1575	From Ox 474 ...	—	—	Never showed trypanosomes.
Average ...			6·0	20·9	

* Duration includes the days of incubation; it dates from day of infection.

II. Strain II., E—.

Table II.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912.					
June 28	764	From Dog 633...	—	—	Never showed trypanosomes.
" 28	765	" 633...	—	—	
" 28	766	" 633...	27 ?	—	Shot "November" 20; broke leg.
Goats.					
June 19	643	From Dog 633...	12	52	Died of Strain II.
" 19	650	" 633...	12	49	" "
" 19	651	" 633...	5	62	" "
" 19	652	" 633...	12	43	" "
		Average ...	10·2	51·5	
Sheep.					
June 28	761	From Dog 633...	10	87	Died of Strain II.
Monkeys.					
June 26	750	From Dog 633...	5	17	Died of Strain II.
" 26	751	" 633...	5	27	" "
" 26	752	" 633...	5	11	" "
" 26	753	" 633...	8	10	" "
		Average ...	5·7	16·2	
Dogs.					
May 29	632	From E— ...	12 ?	30	Died of Strain II.
" 29	633	From E— ...	12 ?	33	" "
June 18	703	From Dog 633...	6	24	" "
" 18	704	" 633...	6	40	" "
		Average ...	6·0	31·8	
Rabbits.					
June 28	762	From Dog 633...	13	33	Died of Strain II.
" 28	763	" 633...	17	33	" "
		Average ...	15·0	33·0	
Guinea-pigs.					
June 19	723	From Dog 633...	—	—	Never showed trypanosomes.
" 19	724	" 633...	—	—	" "
" 19	725	" 633...	—	—	" "
Sept. 7	723	From Sheep 761	12	114	Died of Strain II.
" 7	724	" 761	19	86	" "
" 7	725	" 761	19	84	" "
		Average ...	17·0	94·7	

* Duration includes the days of incubation; it dates from day of infection.

Table II.—*continued.*

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.	
Rats.						
1912.						
June 19	726	From Dog 633...	...	5	43	Died of Strain II.
" 19	727	" 633...	...	8	36	"
" 19	728	" 633...	...	5	52	"
		Average ...	...	6.0	43.7	

III. *Strain III., Chituluka.*

Table III.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912. July 23	967	From Dog 577... ..	20	—	Still alive after 234 days.
„ 23	968	„ 577... ..	83	—	„ „
Goats.					
July 23	937	From Dog 577... ..	13	36	Died of Strain III.
„ 23	938	„ 577... ..	9	19	„ „
„ 23	939	„ 577... ..	9	41	„ „
„ 23	940	„ 577... ..	6	29	„ „
		Average	9.2	31.2	
Monkeys.					
July 23	941	From Dog 577... ..	6	29	Died of Strain III.
„ 23	942	„ 577... ..	6	14	„ „
„ 23	943	„ 577... ..	6	20	„ „
„ 23	944	„ 577... ..	6	11	„ „
		Average	6.0	18.5	
Dogs.					
June 27	577	Chituluka	7	78	Died of Strain III.
July 23	945	From Dog 577... ..	9	31	„ „
„ 23	946	„ 577... ..	6	36	„ „
„ 23	947	„ 577... ..	9	33	„ „
„ 23	948	„ 577... ..	9	33	„ „
		Average	8.0	42.2	

* Duration includes the days of incubation ; it dates from day of infection.

Table III.—*continued.*

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Guinea-pigs.					
1912. July 23	949	From Dog 577... ..	20	62	Died of Strain III.
" 23	950	" 577... ..	20	51	" "
" 23	951	" 577... ..	9	73	" "
		Average	16·3	62	
Rats.					
July 23	952	From Dog 577... ..	6	24	Died of Strain III.
" 23	953	" 577... ..	9	20	" "
" 23	954	" 577... ..	6	21	" "
		Average	7·0	21·7	

IV. Strain IV., *Chipochola*.

Table IV.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912. Sept. 13	1319	From Dog 1260	—	—	Never showed trypanosomes.
" 13	1320	" 1260	—	—	" "
Nov. 8	1319	From Rat 1338	20	—	Still alive after 126 days.
" 8	1320	" 1338	—	—	Never showed trypanosomes.
Goats.					
Sept. 13	1321	From Dog 1260	10	35	Died of Strain IV.
" 13	1322	" 1260	13	59	" "
" 13	1323	" 1260	6	22	" "
" 13	1324	" 1260	24	33	" "
		Average	13·2	37·2	
Monkeys.					
Sept. 13	1325	From Dog 1260	10	16	Died of Strain IV.
" 13	1326	" 1260	10	50	" "
" 13	1327	" 1260	6	51	" "
" 13	1328	" 1260	—	—	Never showed trypanosomes.
		Average	8·7	39·0	

* Duration includes the days of incubation ; it dates from day of infection.

Table IV.—*continued.*

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dogs.					
1912.					
Sept. 4	1260	Chipochola ...	8	32	Died of Strain IV.
" 13	1329	From Dog 1260 ...	6	27	" "
" 13	1330	" 1260 ...	6	27	" "
" 13	1331	" 1260 ...	6	38	" "
" 13	1332	" 1260 ...	6	40	" "
		Average ...	6·4	32·8	
Rabbits.					
Nov. 6	1566	From Rat 1338 ...	5	15	Died of Strain IV.
" 6	1567	" 1338 ...	5	16	" "
		Average ...	5	15·5	
Guinea-pigs.					
Sept. 13	1333	From Dog 1260 ...	—	—	Never showed trypanosomes.
" 13	1334	" 1260 ...	—	—	" "
" 13	1335	" 1260 ...	—	—	" "
Oct. 17	1333	" 1331 ...	11	43	Died of Strain IV.
" 17	1334	" 1331 ...	11	70	" "
" 17	1335	" 1331 ...	11	59	" "
		Average ...	11·0	57·3	
Rats.					
Sept. 13	1336	From Dog 1260 ...	6	26	Died of Strain IV.
" 13	1337	" 1260 ...	6	28	" "
" 13	1338	" 1260 ...	6	93	" "
		Average ...	6·0	49·0	

V. Strain V., Chibibi.

Table V.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Goats.					
1912.					
Nov. 27	1643	From Dog 1599 ...	8	64	Died of Strain V.
" 27	1644	" 1599 ...	8	72	" "
" 27	1645	" 1599 ...	5	41	" "
" 27	1646	" 1599 ...	5	64	" "
		Average ...	6·5	60·2	

* Duration includes the days of incubation ; it dates from day of infection.

Table V.—*continued.*

Date.	Num-ber of experiment.	Source of virus.	Period of incu-bation, in days.	Duration of disease, in days.*	Remarks.
Monkeys.					
1912.					
Nov. 27	1647	From Dog 1599 ...	8	11	Died of Strain V.
" 27	1648	" 1599 ...	—	—	Never showed trypanosomes.
" 27	1649	" 1599 ...	5	21	Died of Strain V.
" 27	1650	" 1599 ...	5	44	" "
		Average ...	6·0	25·3	
Dogs.					
Nov. 14	1599	Chibibi ...	11	44	Died of Strain V.
" 27	1651	From Dog 1599 ...	8	46	" "
" 27	1652	" 1599 ...	5	34	" "
" 27	1653	" 1599 ...	8	45	" "
" 27	1654	" 1599 ...	5	41	" "
1913.					
Jan. 15	1768	From Rat 1744 ...	12	—	Killed January 31.
		Average ...	8·2	42·0	
Rabbits.					
1912.					
Nov. 27	1655	From Dog 1599 ...	8	33	Died of Strain V.
" 27	1656	" 1599 ...	8	23	" "
		Average ...	8·0	28·0	
Guinea-pigs.					
Nov. 27	1657	From Dog 1599 ...	—	—	Never showed trypanosomes.
" 27	1658	" 1599 ...	36	102	Died of Strain V.
" 27	1659	" 1599 ...	—	—	Never showed trypanosomes.
1913.					
Jan. 31	1657	" 1768 ...	—	—	" "
Feb. 10	1657	From Guinea-pig 1658	10	—	Still alive after 43 days.
" 10	1659	" 1658	24	—	" "
		Average ...	23·3	—	
Rats.					
1912.					
Nov. 27	1660	From Dog 1599 ...	5	38	Died of Strain V.
" 27	1661	" 1599 ...	5	30	" "
" 27	1662	" 1599 ...	8	39	" "
1913.					
Jan. 8	1744	From Guinea-pig 1658	6	—	Killed January 22.
" 28	1817	From Monkey 1733 ...	2	20	From Transmission Experiment 1723.
		Average ...	5·2	31·8	Died of Strain V.

* Duration includes the days of incubation ; it dates from day of infection.

Disease set up in Various Animals by the Trypanosome causing Disease in Man in Nyasaland.—I. The Human Strain.

Ox.—This trypanosome is not as deadly to oxen as *Trypanosoma pecorum*; 13 experiments in all were made on cattle with the Human strain. Only one of these died, after an illness lasting 134 days. Four took the disease and recovered, while the remaining eight showed themselves refractory to the injection of blood containing the parasites. One of the recovered animals has been kept under observation for 325 days, and its blood was inoculated into a rat without result. At the present time these so-called recovered animals appear sleek and fat and look healthy, presenting a contrast to the chronic *T. pecorum* infected cattle, which still remain in poor condition. The animal which died was much emaciated, anæmic, and had marked gelatinous infiltration of the connective tissue at the base of the heart and large vessels. The trypanosomes were never numerous in its blood, and in fact only showed six times in the four and a-half months of its illness at the bi-weekly blood examination.

Goat.—This is a fatal disease in goats; 22 were used for experimental purposes, and not one of them recovered. The duration of the disease, on an average, was 41·8 days (19 to 72). One of these animals showed swelling of the face, but none developed opacity of the cornea.

Sheep.—This trypanosome seems as fatal to sheep as goats, killing seven, on an average, in 41·1 days (16 to 87). Among these seven, œdema of the face was noted as a prominent symptom in three. No opacity of cornea developed in any of them.

Baboon.—Only one was inoculated and it proved resistant.

Monkey.—Twenty monkeys died, on an average, in 25·8 days (10 to 51). The trypanosomes were always present in the blood and were often numerous or very numerous. In none of the monkeys was œdema of the face or corneal opacity noted.

Dog.—Twenty-five dogs were inoculated. All died, on an average, in 34·3 days (14 to 78). The parasites were always present on microscopical examination of the blood, and were often numerous and very numerous. In eight of the 25 dogs, opacity of the cornea and swelling of the face were present.

Rabbit.—Seven rabbits died, on an average, in 27·9 days (15 to 42). A rabbit suffering from this disease presents exactly the same clinical picture as that seen in rabbits suffering from Nagana. There is first swelling round the eyes; then the face puffs up, and sores break out round the nose and the eyes. Next there is thickening of the ears, which eventually also become covered with sores exuding a serous fluid. Towards the end the eyes are completely closed up, the nose much swollen, and both eyes and nose discharge a purulent fluid.

Guinea-pig.—These animals are more refractory than rabbits, and often require to be re-inoculated before they take the disease; 15 were used. They died, on an average, in 66·6 days (13 to 114). No prominent symptom, such as œdema or corneal opacity, was seen.

Rat.—Twenty-one were inoculated and died, on an average, in 30·3 days (13 to 93), with their blood swarming with trypanosomes and their spleens enormously enlarged.

COMPARISON OF THE FIVE HUMAN STRAINS OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND, IN REGARD TO THEIR VIRULENCE TOWARDS VARIOUS ANIMALS.

Table VI.—The Average Duration, in Days, of the Disease in Various Animals. The letter R means that the animal is refractory, that is, not susceptible to the disease.

Strain.	Ox.	Goat and sheep.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
I ...	134	34	R	30	24	42	52	21
II ...		59		16	32	33	95	44
III ...		31		18	42		62	22
IV ...		37		39	33	15	57	49
V ...		60		25	42	28	102	32

There would appear to be little difference in the virulence of the five Human strains. If any distinction be made, it might be said that probably Strain I. is the most and Strain V. the least virulent.

Table VII.—The Percentages of Recoveries in Various Animals from the Five Human Strains. The letter R stands for refractory.

Strain.	Man.	Ox.	Goat and sheep.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
I ...	0	50	0	R	0	0	0	0	0
II ...	0	?	0		0	0	0	0	0
III ...	0	100	0		0	0	0	0	0
IV ...	0	100	0		0	0	0	0	0
V ...	0		0		0	0	0	0	0

It would appear to be equally impossible to separate the Human strains by the percentages of recoveries. All the experimental animals, except the ox, succumb to the disease if once the parasite has obtained a footing.

Table VIII.—The Average Duration of Life, in Days, of Various Animals infected with the Human Strain. The letter R stands for refractory.

—	Ox.	Goat and sheep.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days.	134	42	R	26	34	28	67	30
Number of animals employed.	1	29	1	20	25	7	15	21

This table shows the extreme virulence of this trypanosome for most animals except the ox. The guinea-pig is somewhat refractory, and often resists the first injection of infected blood, but not the second.

Table IX.—The Percentage of Recoveries in Various Animals infected with the Five Human Strains. The letter R stands for refractory.

—	Man.	Ox.	Goat and sheep.	Ba- boon.	Mon- key.	Dog.	Rab- bit.	Guinea- pig.	White rat.
Percentages ...	0	80	0	R	0	0	0	0	0
Number of ani- mals employed.	5	5	29	1	20	25	7	15	21

Conclusions.

1. The trypanosome causing disease in man in Nyasaland is fatal to goats, sheep, dogs, and the smaller laboratory animals, killing them, without exception, in a few weeks. It is less virulent to cattle, many of which evidently escape.

2. No difference in virulence can be made out in these five Human strains.

3. It is not satisfactorily proved yet to what species this trypanosome belongs, but the Commission at present leans to the opinion that it is *T. brucei* (Plimmer and Bradford).

XII.—TRYPANOSOMES OF THE DOMESTIC ANIMALS IN NYASALAND. I. *TRYPANOSOMA SIMLÆ*, SP. NOV. PART II.—THE SUSCEPTIBILITY OF VARIOUS ANIMALS TO *T. SIMLÆ*.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.;
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C.; and Lady BRUCE, R.R.C.

(Received June 2, 1913.)

INTRODUCTION.

In a previous paper† the morphology of this interesting species of trypanosome was described, and it is now proposed to give an account of its action on animals.

* Reprinted from the Proceedings of the Royal Society, B, Vol. 87.

† 'Roy. Soc. Proc.', 1912, B, Vol. 85, pp. 477-481.

One of the first interesting points to be noted about this species is that, as far as is known, the warthog (*Phacochærus æthiopicus*) is the only animal among the wild game of this district which harbours it.* It is probable that it will also be found in the blood of the bush-pig, but not a single specimen of this animal has as yet been obtained by the Commission. The warthog is numerous in the low country in this neighbourhood, which accounts for the large number of tsetse flies found to be infected with *Trypanosoma simia*.†

It is to be regretted that this species was not named after the warthog instead of the monkey, but at the time the name *Simia* was taken the Commission was ignorant of the close connection which exists between the former animal and this parasite.

Another interesting feature in regard to this trypanosome is the virulence it displays towards monkeys and the domestic pig, killing these animals in an incredibly short period of time, whereas it is harmless to oxen, antelope, dogs, and the smaller experimental animals. Curiously enough, this trypanosome also infects goats and sheep, although oxen and antelope escape.

The rapidity with which the virulence of *T. simia* becomes modified is also remarkable. When a cage containing wild *Glossina morsitans* is placed on a monkey or a goat, both animals take the disease, and the donkey in such an acute form that the average duration of life is only a few days. But if it is attempted to pass *T. simia* from an infected goat to a healthy monkey by the inoculation of the goat's blood, the experiment usually fails, showing that a short sojourn in the blood of the goat has almost nullified the virulence of the parasite for the monkey.

T. simia belongs to the same group as *T. pecorum*, and it is curious that in the latter species this loss of virulence also occurs. If *T. pecorum*, which is usually more or less infective to the monkey, dog, and rat, lives for some time in the blood of the goat, it loses its power of infecting the other animals. This has given rise to the erroneous idea that a separate species—*T. nanum*—exists. *T. nanum* is in truth nothing but a strain of *T. pecorum* which has lost its virulence for these other animals by its passage through the goat.

T. simia is also like *T. pecorum* in general appearance, and in fact it is often difficult or impossible to distinguish between a short individual of the former species and a long one of the latter. The average length of *T. simia* is 17·5 microns, with a minimum of 14; the average length of *T. pecorum* is 14 microns, with a maximum of 18. When the coloured plates of the two species are compared this resemblance is at once seen. There is the same well-developed undulating membrane, the same oval nucleus, situated about the middle of the body, and the same eccentrically-placed micronucleus, often appearing to project beyond the margin, as noted in the description of the morphology of *T. simia*.

These two species, however, differ essentially in their action on animals. Whereas *T. simia* expends its virulence on monkeys and pigs, *T. pecorum* is especially fatal to cattle, goats, sheep, and dogs.

* 'Roy. Soc. Proc.,' 1912, "Trypanosomes found in Blood of Wild Animals."

† *Ibid.*, "Infectivity of *Glossina morsitans* in Nyasaland."

ANIMALS SUSCEPTIBLE TO *TRYPANOSOMA SIMILÆ*, SP. NOV.

Table I.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912.					
April 24	485	From Monkey 405	—	—	Never showed trypanosomes.
" 24	486	" 405	—	—	" "
July 17	904	From Goat 653	...	—	" "
" 17	945	" 653	...	—	" "
Antelope.					
Sept. 25	1240	From Monkeys 1258 and 1259.	—	—	Duiker. Never showed trypanosomes.
Nov. 5	1238	From Monkey 1541	—	—	Reedbuck. Never showed trypanosomes.
" 5	1240A	" 1541	—	—	Duiker. Never showed trypanosomes.
1913.					
Mar. 5	1980	" 1910	—	—	Reedbuck. Never showed trypanosomes.
" 5	1981	" 1910	—	—	Duiker. Never showed trypanosomes.
Goats.					
1912.					
Feb. 1	109	Wild flies ...	17	55	Mixed infection.
" 1	117	From Monkey 20...	11	50	" "
" 3	125	Wild flies ...	7	36	" "
" 9	175	" ...	20	88	" "
" 19	247	" ...	21	30	" "
" 27	262	" ...	9	—	Still alive after 398 days.
Mar. 23	363	" ...	6	42	Mixed infection.
April 19	428	" ...	6	13	Died of <i>T. similæ</i> .
" 24	425	From Monkey 405	22	—	Still alive after 341 days.
" 24	426	" 405	—	—	Never showed trypanosomes.
" 29	429	Wild flies ...	12	42	Mixed infection.
May 14	279	" ...	16	54	Died of <i>T. similæ</i> .
" 15	416	" ...	5	19	Mixed infection.
" 18	553	" ...	11	112	" "
June 1	571	" ...	11	23	" "
" 4	620	" ...	3	30	Died of <i>T. similæ</i> .
" 19	653	From Goat 620	8	29	" "
" 19	654	" 620	8	107	" "
" 19	710	" 620	8	50	Mixed infection.
Aug. 21	1117	From Warthog 1139	40	—	Still alive after 222 days.
" 24	1113	" 1186	23	—	" " 219 "
Sept. 12	1311	" 1308	18	—	Killed October 3.
" 20	1461	From Goat 1113	9	—	Still alive after 183 days.
Oct. 3	1470	" 1311	21	—	" " 179 "
" 14	1483	" 1461	14	—	" " 168 "
Nov. 5	1550	From Monkey 1541	6	—	" " 146 "
" 5	1551	" 1541	6	—	" " 146 "
" 5	1552	" 1541	6	—	" " 146 "
" 15	1601	From Pig 1585	3	—	" " 136 "
" 20	1613	Wild flies ...	9	—	" " 131 "

* Duration includes the days of incubation ; it dates from day of infection.

Table I.—continued.

Date.	Number of experiment	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Goats—continued.					
1913. Jan. 27	1810	Wild flies ...	6	52	Mixed infection.
Sheep.					
1912. July 11	907	From Goat 653 ...	5	36	Mixed infection.
Pigs.					
Nov. 5	1553	From Monkey 1541	6	6	Died of <i>T. simia</i> .
" 5	1554	" " 1541	6	6	" "
" 5	1555	" " 1541	6	7	" "
" 11	1585	From Pig 1555 ...	4	5	" "
" 15	1600	" " 1585 ...	3	4	" "
" 18	1609	" " 1600 ...	3	4	" "
" 18	1611	Wild flies ...	3	7	" "
" 23	1631	From Goats 1113, 1434, 1461, 1470, and 1483.	—	—	Never showed trypanosomes.
" 25	1636	Wild flies ...	13	98	Mixed infection.
Dec. 3	1665	" ...	6	17	" "
" 6	1674	" ...	3	10	" "
" 11	1683	" ...	3	5	Died of <i>T. simia</i> .
" 16	1701	" ...	6	12	Mixed infection.
1913. Jan. 24	1801	" ...	3	4	Died of <i>T. simia</i> .
Baboons.					
1912. April 30	499	From Monkey 449	—	—	Never showed trypanosomes.
" 30	500	" 449	—	—	" "
" 30	501	" 449	—	—	" "
Monkeys.					
Jan. 20	20	Wild flies ...	7	9	Died of <i>T. simia</i> .
" 24	55	" ...	9	9	" "
" 29	54	" ...	5	12	" "
Feb. 2	49	" ...	6	8	" "
" 6	59	From Monkey 55 ...	6	8	" "
" 8	58	Wild flies ...	11	—	Still alive after 318 days.
" 13	219	" ...	7	9	Mixed infection.
" 27	286	From Dog 211 ...	6	52	" "
Mar. 9	326	From Goat 175 ...	—	—	Never showed trypanosomes.
April 15	405	Wild flies ...	5	—	Killed April 24.
" 18	447	" ...	9	—	Still alive after 159 days.
" 18	448	" ...	8	12	Died of <i>T. simia</i> .
" 19	404	" ...	5	6	" "

* Duration includes the days of incubation ; it dates from day of infection.

Table I.—continued.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Monkeys—continued.					
1912.					
April 19	449	From Monkey 404	3	10	Died of <i>T. simia</i> .
" 23	465	Wild flies ...	4	8	" "
" 24	480	From Monkey 405	8	10	" "
" 26	488	Wild flies ...	10	13	" "
" 27	492	From Dog 436	5	63	Mixed infection.
" 27	495	Wild flies ...	7	11	Died of <i>T. simia</i> .
May 3	504	" ...	12	11	" "
" 8	523	" ...	4	5	" "
" 9	521	" ...	5	7	" "
" 14	545	" ...	3	6	" "
" 24	576	From Monkey 545	10	14	" "
" 31	601	Wild flies ...	6	31	Mixed infection.
June 11	629	" ...	11	17	Died of <i>T. simia</i> .
July 17	906	From Goat 653	8	11	" "
Sept. 18	1404	" 1113	—	—	Never showed trypanosomes.
" 18	1405	" 1113	—	—	" "
" 28	1404	" 1113	—	—	" "
" 28	1405	" 1113	—	—	" "
Oct. 3	1468	" 1311	—	—	" "
" 3	1476	" 1117	—	—	" "
" 14	1404	" 1461	—	—	" "
" 14	1405	" 1461	—	—	" "
" 23	1515	Wild flies ...	6	8	Died of <i>T. simia</i> .
" 29	1404	From Goat 1483	—	—	Never showed trypanosomes.
" 29	1405	" 1483	—	—	" "
" 29	1535	" 1483	—	—	" "
Nov. 1	1541	From Monkey 1515	3	—	Killed " for inoculation purposes.
" 1	1542	" 1515	3	11	Died of <i>T. simia</i> .
" 11	1583	From Pig 1555	3	20	" "
" 11	1584	" 1555	3	—	Still alive after 140 days.
" 11	1586	Wild flies ...	7	12	Died of <i>T. simia</i> .
" 19	1614	From Goat 1601	—	—	Never showed trypanosomes.
" 19	1617	" 1550	—	—	" "
" 27	1663	" 1601	8	—	Still alive after 124 days.
" 28	1404	From Monkey 1586	—	—	Never showed trypanosomes.
Dec. 7	1677	From Goat 1550	—	—	" "
" 11	1681	Transmission experiment.	11	23	Died of <i>T. simia</i> .
" 14	1697	From Goat 1551	—	—	Never showed trypanosomes.
" 14	1698	" 1601	—	—	" "
1913.					
Jan. 16	1772	Wild flies ...	14	—	Still alive after 74 days.
		Average ...	7.6	10.8	

* Duration includes the days of incubation; it dates from day of infection.

Table I.—*continued.*

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dogs.					
1912.					
Feb. 6	119	From Monkey 55...	—	—	Never showed trypanosomes.
" 6	128	" 55...	—	—	" "
" 6	130	" 55...	—	—	" "
" 16	211	Wild flies ...	—	—	" "
" 24	250	From Monkey 219	—	—	" "
" 24	251	" 58	—	—	" "
" 24	254	" 58	—	—	" "
" 24	258	" 219	—	—	" "
Mar. 9	320	From Goat 175 ...	—	—	" "
" 9	321	" 125 ...	—	—	" "
" 9	319	" 175 ...	—	—	" "
" 9	322	" 125 ...	—	—	" "
" 9	325	From Monkey 286	—	—	" "
" 13	330	" 58	—	—	" "
April 5	409	" 58	—	—	" "
" 12	436	Wild flies ...	—	—	" "
" 24	481	From Monkey 405	—	—	" "
" 24	482	" 405	—	—	" "
" 27	489	" 449	—	—	" "
" 27	490	" 449	—	—	" "
Oct. 3	1469	From Goat 1311 ...	—	—	" "
" 29	1520	" 1483 ...	—	—	" "
Rabbits.					
Oct. 29	1522	From Goat 1483 ...	—	—	Never showed trypanosomes.
" 29	1523	" 1483 ...	—	—	" "
Nov. 5	1543	From Monkey 1541	—	—	" "
" 5	1544	" 1541	—	—	" "
" 5	1545	" 1541	—	—	" "
Dec. 14	1543	From Pig 1636 ...	—	—	" "
" 14	1544	" 1636 ...	—	—	" "
" 14	1545	" 1636 ...	—	—	" "
" 24	1714	Wild flies ...	7	11	Mixed infection.
1913.					
Jan. 28	1827	" ...	9	—	Mixed infection. Still alive after 62 days.
Guinea-pigs.					
1912.					
April 24	483	From Monkey 405	—	—	Never showed trypanosomes.
Oct. 29	1524	From Goat 1483 ...	—	—	" "
Nov. 5	1546	From Monkey 1541	—	—	" "
" 5	1547	" 1541	—	—	" "
1913.					
Jan. 4	1731	Transmission experiment.	—	—	" "

* Duration includes the days of incubation ; it dates from day of infection.

Table I.—continued.

Date.	Number of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Rats.					
1912.					
April 24	484	From Monkey 405	—	—	Never showed trypanosomes.
" 27	491	" 449	—	—	" "
Oct. 29	1521	From Goat 1483 ...	—	—	" "
Nov. 5	1548	From Monkey 1541	—	—	" "
" 5	1549	" 1541	—	—	" "

Action of T. simiæ on Horses, Oxen, and Antelope.—There has been no opportunity of testing the action of *T. simiæ* on equines. Four oxen were inoculated, two from an infected monkey and two from a goat, but all four remained in good health. Five antelope in confinement were also inoculated without result, and it would seem that these animals were really refractory, since in no instance has *T. simiæ* ever been found in the blood of antelope.

Disease set up in Goats and Sheep by T. simiæ.—Thirty-one goats and one sheep were infected by this parasite, as the result of various experiments. Fifteen of these were used in wild *G. morsitans* feeding experiments, three had warthog blood injected into them, and 14 were inoculated with the blood of infected experimental animals. It has already been remarked that this species of trypanosome varies rapidly in its virulence or power of setting up disease in animals. Its virulence would appear to be exalted by passage through the "fly," or, at least, to have reached its highest virulence after passage through the "fly," and lowered by passage through certain animals. For example, of the 15 goats infected by the bite of the "fly," 13 died, on an average, in 46 days, and only two recovered. The three goats which were infected by the direct injection of warthog blood all recovered; this is curious when it is remembered that in all probability the "fly" must get its infection solely from this animal. Six goats were injected with the blood of "fly"-infected monkeys; only one died, four recovered, and one proved refractory. Six goats were also infected from goats;

* Duration includes the days of incubation; it dates from day of infection.

of these, three died and three recovered. This variation in virulence may also be expressed, for the sake of clearness, in the following table:—

Table II.—Mortality among Goats infected in Various Ways by *T. simia*.

	Mode of Infection.			
	Wild <i>G. morsitans</i> feeding.	Warthog blood, injection of.	Monkey blood, injection of.	Goat blood, injection of.
Percentage of deaths ...	86·7	0·0	16·7	50·0
Number of goats employed	15	3	6	6

What does this table show? It is meant to show that if goats are infected with *T. simia* directly by the bite of the “fly,” most of them will die, whereas if the parasite is passed for a generation or two through goats, monkeys, or the warthog, the rate of mortality for the goat will fall. But it may be objected that the six goats which were infected with goat’s blood have a mortality of 50 per cent., which is almost as large as the wild *G. morsitans* feeding experiments. But if Table I. be referred to, it will be found that the three fatal cases were inoculated with blood from a goat which had only recently—some 10 days—been infected by the bite of the “fly,” and ought, on account of the shortness of the time, to be included in the first, or “fly”-feeding, column; the other three, non-fatal cases, from goats infected by the injection of warthog’s blood, should be included in the second column. If this were done, then the table would appear as follows:—

Table III.

	Mode of Infection.		
	Wild <i>G. morsitans</i> feeding.	Warthog blood, injection of.	Monkey blood, injection of.
Percentage of deaths ...	90·0	0·0	16·7
Number of goats employed	18	6	6

It must be confessed, however, that the numbers are small and subject to a large margin of error, but the figures seem sufficiently remarkable to merit record. It may also be remarked that the matter is of little practical importance, as under natural conditions goats will always be infected by the bite of the “fly”; but it is interesting as showing the fallacy of judging from the action of laboratory strains as to what will occur in nature.

Among the goats infected by wild *G. morsitans*, there are many cases of mixed infection. On referring to Table I. it will be seen that there are 15 cases of infection by wild *G. morsitans* among the goats. Of these, only three were cases of pure infection with *T. simiæ*, the remaining 12 were mixed infections. These three died, on an average, in 32 days. There were 12 cases of mixed infection; of these, one recovered and the remaining 11 died, on an average, in 49 days.

From this it would appear that *T. simiæ*, acting alone, is as rapidly fatal to goats as it is when occurring in various combinations with *T. brucei*, *T. pecorum*, or *T. capræ*. *T. simiæ* infection, when the result of the bite of the "fly," must therefore be looked upon as a fairly deadly enemy to goats.

During life this disease of goats is not marked by any special symptoms. There are neither swellings of the body, limbs, or face, nor corneal opacities, as is sometimes the case in other trypanosome diseases of goats: the animal merely becomes more and more anæmic and emaciated, and finally dies of exhaustion.

Disease set up in the Domestic Pig by T. simiæ.—In the whole range of the trypanosome diseases of animals there is surely nothing so striking as the rapidly fatal action of *T. simiæ* on the domestic pig. In nine experiments the average duration was only 5·3 days. This, not from the time of the appearance of the trypanosomes in the blood, but from the date of infection. Further, this rapid action is not the result of an exaltation of virulence by numerous passages through the pig, but natural to the trypanosome.

In regard to the symptoms of the disease during life, nothing noteworthy happens owing to the rapidity of the disease.

Disease set up in the Monkey by T. simiæ.—This trypanosome is also remarkably fatal to the monkey. In 20 experiments with wild *G. morsitans* 17 monkeys died, the duration, on an average, being only 9·5 days from the day the flies were fed. Three are still alive after 318, 159, and 74 days, and have evidently recovered. When a monkey is inoculated with blood from an infected monkey the result is the same, the duration in five cases being 10·6 days. It will be seen, however, from Table I., that these five monkeys were all inoculated with blood from monkeys which had only very recently been infected by the bite of the "fly." On the other hand, when a monkey is inoculated with blood from a goat infected with *T. simiæ*, the result, as a rule, is negative. Ten monkeys were inoculated with blood of "fly"-bitten goats and only one became infected; the other nine remained negative although the trypanosome had only been in the goats an average of 23 days. From this it would appear that *T. simiæ* loses its virulence for the monkey if exposed to the action of the living goat's blood for even so short a period as 12 or 14 days.

Action of T. simiæ on the Dog.—The dog appears to be immune to this species of trypanosome. Many experiments were made by feeding wild *G. morsitans* on dogs and by inoculating the blood of infected goats and monkeys. In not a single case did the trypanosomes appear in the blood nor did the dogs appear to be affected in any way. But in two "fly"-feeding experiments

(211 and 436), although *T. simia* did not appear in the blood of the dogs on microscopical examination, yet the injection of their blood into monkeys gave rise to an infection with *T. simia*, showing that the parasite was present although in numbers too small to be detected by the microscope. It is possible, then, that the dog may act as a reservoir of this disease, but most improbable that it does so to any practical extent.

Action of T. simia on Rabbits.—The rabbit seems also to be practically immune to this disease. The injection of blood from infected goats, pigs, and monkeys has no effect. But on two occasions after feeding wild *G. morsitans* on rabbits, *T. simia* appeared in their blood, in one case (Experiment 1714) in large numbers, in the other (Experiment 1827) only rarely. Both these were examples of mixed infection, the former of *T. simia* and *T. pecorum*, the latter of *T. simia*, *T. pecorum*, and *T. brucei*. It was attempted to infect a rabbit by feeding on it a fly which was known to be infective with pure *T. simia*, but with no result.

This susceptibility, or non-susceptibility, of rabbits to *T. simia* is perhaps not a very important matter, but the experiments go to show that the natural mode of infection of trypanosome diseases by means of the "fly" is probably the most effective.

Action of T. simia on Guinea-pigs and Rats.—Both these species of animals appear to be refractory.

Table IV.—The Average Duration of Life in Various Animals infected by *T. simia*, Nyasaland. Mixed infections are not included. The duration includes the days of incubation; it dates from the date of infection. The letter R stands for refractory.

—	Ox.	Antelope.	Goat and sheep.	Pig.	Ba-boon.	Mon-key.	Dog.	Rabbit.	Guinea-pig.	Rat.
Average duration in days.	R	R	46·6	5·3	R	10·8	R	R	R	R
No. of animals employed.	4	5	5	9	3	24	21	10	5	5

Table V.—The Percentages of Recoveries in Various Animals from *T. simia* infection. This table includes mixed infections.

—	Ox.	Antelope.	Goat and sheep.	Pig.	Ba-boon.	Mon-key.	Dog.	Rabbit.	Guinea-pig.	Rat.
Percentages...	R	R	37·5	0·0	R	14·3	R	R	R	R
No. of animals employed.	4	5	32	13	3	35	21	10	5	5

THE CARRIER OF *T. SIMIÆ*, NYASALAND.

In Nyasaland the carrier of *T. simiæ* is *G. morsitans*, of which 3·4 per 1,000 were found to be infected. A paper on the development of *T. simiæ* in *G. morsitans* is in course of preparation.

THE HOST, OR RESERVOIR, OF *T. SIMIÆ*.

The warthog.—Thirty-three of these animals were examined, and *T. simiæ* found in three.

Conclusions.

1. *T. simiæ* belongs to the same group as *T. pecorum*, and, like the latter is erratic in its action on animals.
2. *T. simiæ* affects goats, sheep, pigs, and monkeys. Oxen, antelope, dogs, rabbits, guinea-pigs, and rats are practically immune.
3. The carrier is *G. morsitans*.
4. The reservoir of the virus is the warthog.

XIII. — TRYPANOSOME DISEASES OF DOMESTIC ANIMALS IN NYASALAND. I.—*TRYPANOSOMA SIMIÆ*, SP. NOV. PART III.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.,
Majors DAVID HARVEY and A. E. HAMERTON, D.S.O.,
R.A.M.C., and Lady BRUCE, R.R.C.

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[PLATES 8-10.]

INTRODUCTION.

In previous papers† the morphology of *Trypanosoma simiæ* and its action on animals have been described. In this it is intended to give an account of its development in *Glossina morsitans*.

Before entering, however, into the details of this particular development, it may be of interest to take a general survey of the various modes of development which take place in the different groups of trypanosomes. It may then be laid down

* Reprinted from the Proceedings of the Royal Society, B, Vol. 87.

† 'Roy. Soc. Proc.,' 1912, B, Vol. 85, and 1913, B, Vol. 86.

that there is a well marked separate and characteristic mode of development in each of the three main groups of trypanosomes:—

In the first group—the *T. brucei* group—which includes *T. brucei*, *T. gambiense*, *T. evansi* (?), and *T. equiperdum* (?), the parasites develop—at least in the first two named species—at first through the whole length of the intestinal tract, excluding the proboscis, and eventually reach the salivary glands, where forms resembling those found in the blood of animals are developed, and these alone constitute the infective stage.

In the second group—the *T. pecorum* group—which includes *T. pecorum* and *T. simia*, the development takes place in the intestinal tract, including the labial cavity of the proboscis, and afterwards the trypanosomes reach the hypopharynx, or termination of the salivary duct in the proboscis. Here they revert to the original blood form and become infective. In this group trypanosomes are never found in the salivary glands, and no blood forms or infective forms are developed until the hypopharynx is reached.

In the third group—the *T. vivax* group—which includes *T. vivax*, *T. uniforme*, and *T. caprae*, the initial stages of the development takes place in the labial cavity of the proboscis alone; later the hypopharynx is invaded, where again blood forms are developed, which again constitute the only infective forms. Here also there is no invasion of the salivary glands, and, in addition, no development takes place in the intestinal canal.

In all three groups the common factor which leads to the formation or development of the final or infective forms is the invasion of the salivary tract, and this is accompanied by a reversion to the original blood forms.

DEFINITIONS.

In this paper the word “proboscis” will mean the piercing apparatus of the “fly,” made up of the labrum, labium, labellum, and hypopharynx. There are two tubes in the proboscis; one for the passage inwards of blood, made up by the coalition of the labrum and labium, the other for the passage outwards of the salivary secretion—the terminal salivary duct or hypopharynx. The term “labial cavity” in this paper will mean the former, or tube for conveyance of blood, the word “hypopharynx” the latter, or duct for conveyance of saliva. In the past the use of the word “proboscis,” including both tubes, has given rise to a good deal of ambiguity.

The definition of the words “infected” and “infective” were given in a previous paper.*

The term “blood form” means a stage in the development of the trypanosomes in the “fly,” when there is a reversion to the original form found in the blood of animals, and from which the cycle of development originated.

* ‘Roy. Soc. Proc.’ B, Vol. 86. (“Infectivity of *Glossina morsitans* in Nyasaland.”) *Supra*, p. 73.

THE DEVELOPMENT OF *T. SIMILE* IN *G. MORSITANS*.

Eight experiments were carried out with laboratory-bred flies. Two were positive and six were negative. The following table shows these eight experiments, the number of flies used, the number of infected flies found on dissection and the number of days which elapsed before the flies became infective:—

Table I.

Date.	Experiment.	Number of flies used.	Experiment positive or negative.	Number of infected flies found.	Number of days before flies became infective.
1912.					
May 1 ...	502	20	—		50
June 27 ...	754	31	+	2	
Oct. 9 ...	1477	17	—	1	
Nov. 11 ...	1582	8	—		
" 15 ...	1602	20	—		
" 21 ...	1622	16	—		
1913.					
Feb. 5 ...	1847	45	+	7	20?
" 10 ...	1856	16	—		

It will be noted that there is a great difference between the two positive experiments as regards the time required for the flies to become infective. In the first 50 days elapsed, in the second only 20. This is due to different temperatures under which the experiments were carried out. The first positive experiment was done during the coldest time of the year on Kasu Hill, when the mean temperature was 62° F. (16·6° C.), which is much lower than on the plains, the natural habitat of *G. morsitans*. The flies in the other experiment were kept in an incubator at a temperature of 83° F. (28·3° C.), and they became infective much sooner.

Details of the Two Positive Experiments.

The following table gives the principal details of the first positive experiment:—

Experiment 754.

Table II.

Day of experiment.	Procedure.	Remarks.
1-3	Flies fed on <i>T. simiae</i> -infected monkey.	Trypanosomes first appeared on the 57th day.
4	Starved.	
5-60	Fed on clean monkey.	

It is seen that it was not until the flies had been fed on the clean monkey for 57 days that the animal showed trypanosomes in its blood. If we allow seven days for the average incubation period of the parasite in the mammalian host, then the monkey contracted the disease about the 50th day after the infecting fly had fed on trypanosome-infected blood.

The following table gives the principal details of the second positive experiment:—

Experiment 1847.

Table III.

Day of experiment.	Procedure.	Remarks.
1-10	Flies fed on <i>T. simia</i> -infected monkey.	Flies became infective on the 26th day after first infected feed ; 16 days after the last.
11	Starved.	
12-27	Fed on clean monkey.	

Since the flies of this experiment were fed on infected blood for a period of 10 days, the time required for the trypanosomes taken up by the "fly" to multiply and regain their virulence cannot be accurately estimated. Allowing seven days for the incubation period it cannot be more than 20 days.

When the healthy monkey became infected, in order to separate the infective flies, those remaining alive were divided into three batches. Each batch was put into a cage and fed separately on a healthy monkey. The following table gives the details and results of feeding the three batches of flies:—

Table IV.

Experiment.	Batch.	Number of flies.	Number of days fed.	Result.	Number of infected flies found.
1847	1	12	7	—	0
1847	2	10	7	+	3
1847	3	13	7	+	3

The monkeys on which Batches 2 and 3 were fed showed trypanosomes in their blood on the sixth day after the first application of the flies. It is therefore highly probable that the flies infected the monkeys on the first day of feeding.

Details of the Six Negative Experiments.

The following table shows the method of procedure in carrying out the six negative experiments:—

Table V.

Experiment.	Day of experiment.	Procedure.	Remarks.
502	1-2	Fed on infected monkey.	All flies negative on dissection.
	3-4	Starved.	
	5-42	Fed on clean monkey.	
1477	1-3	Fed on infected goat.	One infected fly found on the 40th day; proboscis and gut infected.
	4-5	Starved.	
	6-45	Fed on clean monkey.	
1582	1st	Fed on infected pig.	All flies negative on dissection.
	2nd	Starved.	
	3-30	Fed on clean monkey.	
1602	1st	Fed on infected pig.	All flies negative on dissection.
	2nd	Starved.	
	3-35	Fed on clean monkey.	
1622	1st	Fed on infected pig.	All flies negative on dissection.
	2nd	Starved.	
	3-29	Fed on clean monkey.	
1856	1-7	Fed on infected monkey.	All flies negative on dissection.
	8-26	Fed on clean monkey.	

In Experiment 1477 a portion of the intestine of the infected fly was inoculated subcutaneously into a pig; the pig did not become infected.

Out of a total of 173 flies used in these experiments, 10 flies (5·8 per cent.) became infected with a growth of trypanosomes in the intestines and in the probosces. It will also be seen that only 1 fly in 31 (2·7 per cent.) became infective when the flies were kept at ordinary room temperature, whereas 4 became infected in 45 (9 per cent.) when the flies were kept at a temperature of 28° C.

GENERAL CONSIDERATIONS REGARDING THE DEVELOPMENT OF
T. SIMLÆ IN G. MORSITANS.

All the flies dying during the progress of the experiments were dissected. In the two positive experiments, out of 76 flies

dissected, nine infected flies were found. The following table gives the results of the dissection of these nine flies:—

Table VI.

Ex- peri- ment.	Time, days.	Proboscis.		Pro- ventri- culus.	Crop.	Fore- gut.	Mid- gut.	Hind- gut.	Proc- to- dæum.	Sali- vary glands.
		Labial cavity.	Hypo- pharynx.							
754	37	++		++	—	++	++	—	—	—
754	50	++		++	—	++	++	—	—	—
1847	16-26	—	—	—	—	++	++	—	—	—
1847	30-40	+	+	—	—	++	++	—	—	—
1847	31-41	++	+	++	—	++	++	++	—	—
1847	31-41	++	—	+	—	++	++	++	—	—
1847	31-41	++	+	+	—	++	++	—	—	—
1847	32-42	++	+	+	—	++	++	++	—	—
1847	32-42	—	—	+	—	++	++	—	—	—

From this table it will be seen that in seven out of nine flies dissected the labial cavity is found to contain trypanosomes. This is very different from what is seen in the similar table relating to *T. gambiense*. There not a single case of infection of the proboscis is recorded.*

At what stage in the development of the trypanosome the proboscis takes a part is not known. It is probable that the infection commences in the intestinal tract and moves forward into the proboscis, but owing to the difficulty of obtaining sufficient laboratory-bred *G. morsitans* the Commission have not, up to the present, enough evidence to establish this detail.

In the two infected flies found in the cage of flies, Experiment 754, it is to be regretted that the contents of the hypopharynx were not noted, but in all the infected flies found in Experiment 1847 this was done, with the result that the hypopharynx was found invaded by trypanosomes in four out of the seven.

Plate 8 represents, at a magnification of 500 diameters, the labial cavity and hypopharynx of an infected fly. While the labial cavity contains clusters of large ribbon-like trypanosomes, the hypopharynx is swarming with small active forms resembling the original blood forms, from which the developmental cycle arose. When the plate is examined the facility with which a tsetse fly can infect an animal will no longer be a matter of wonder.

Finally, from the table it will be seen that in no case were the salivary glands invaded.

* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 516. (S.S. Report XI, p. 37.)

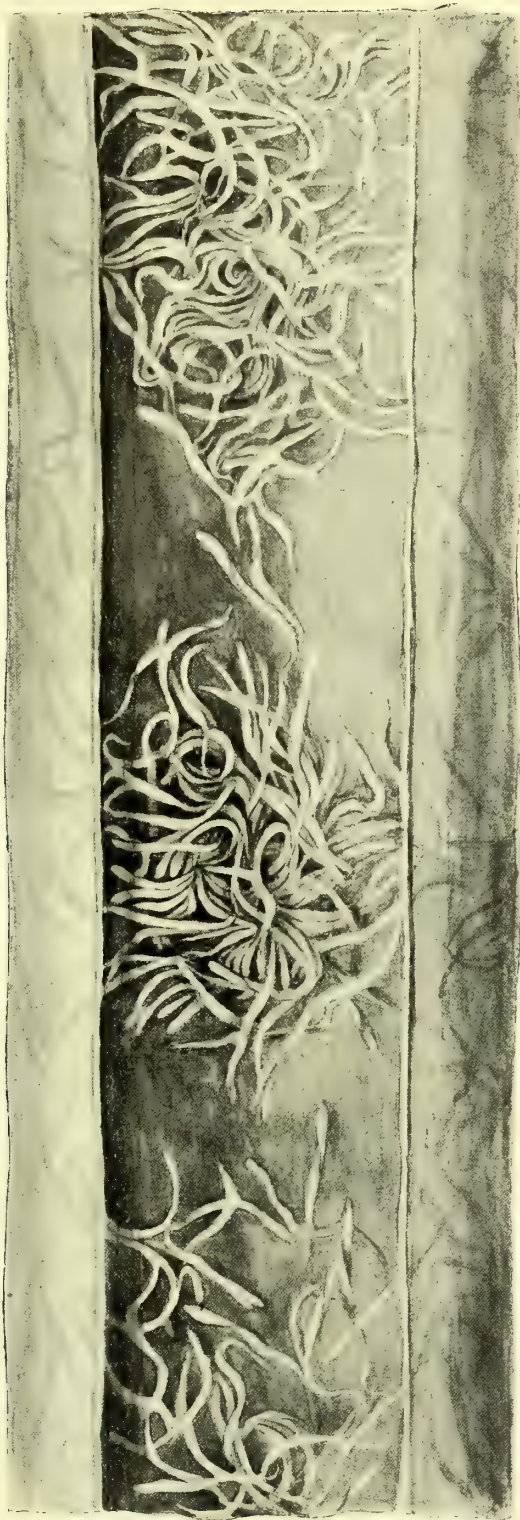


FIG. 1.

T. simiae in labrum and hypopharynx

M. E. Bruce, del.



FIG. 2.

× 750.

[To face page 136.]



THE METHODS USED IN THE EXAMINATION OF THE FLIES.

The flies were dissected as described in a previous paper.* An additional method of examining the contents of the hypopharynx was to isolate infective flies by putting each fly into a separate tube, numbering it, and feeding the fly on a susceptible animal with a corresponding number on its cage. The numbers on the cages of animals which became infected indicated the tubes containing infective flies. These, when thus identified, were starved for 24 hours, in order to make them hungry. A tube containing one of the infective flies was then taken, and its mouth being covered with mosquito netting was applied to a large cover-glass placed on a man's finger. The hungry fly at once attempted to feed through the glass, and in poking about with its proboscis smeared the surface of the cover-glass with saliva. This was immediately fixed, stained with Giemsa, and examined.

THE TRYPANOSOMES FOUND IN THE PROBOSCIS.

Reference to the table above will show that in Experiment 754 two infected flies were found, one on the 37th day after feeding on an infected monkey, and one on the 50th day, and that the labial cavities of both flies were infected.

The fly that died on the 50th day was the one which no doubt actually infected the healthy monkey, since the animal showed trypanosomes seven days after the death of this fly and no other infected fly was found. As these two flies died before they were isolated, the method of inducing them to salivate on a cover-glass was not used. When, however, the two proboscides were examined in a drop of normal saline solution under a cover-glass, trypanosomes attached to the labrum were seen growing in colonies in the labial cavity. They were moving freely and some detached individuals were swimming actively up and down the lumen of the tube.

It is to be regretted that the contents of the hypopharynx were not specially noted. These were two of the earliest experiments, and at that time the contents of the labial cavity and the hypopharynx were not differentiated.

In Experiment 1847 seven infected flies were found. It will be observed (Table VI.) that the first was dissected on the 16th day after the last infected feed and that the proboscis was not infected. Another fly dissected on the 32nd day had also no infection of the proboscis. A third fly dissected on the 31st day had the labial cavity of the proboscis infected but not the hypopharynx. The remaining four were found to contain swarms of trypanosomes in both the labial cavity and the hypopharynx. On examination it was observed that there were two distinct

* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 513. (S.S. Report XI., p. 34, &c.)

varieties. One found in the hypopharynx closely resembled small blood forms of *T. simia* (Plate 10, figs. 18 to 21). They swarmed in the narrow tube, which had the appearance of being blocked up by their enormous numbers. These small blood forms of the parasite were readily distinguishable from those growing in the labial cavity. Those growing in the labial cavity resemble *Leptomonas*, and are peculiar in having their non-flagellated extremity prolonged to a snout-like extension (Plate 10, figs. 12 to 16). They are assembled in clusters and attached by their flagella to the inner surface of the labrum, their prolonged free extremity moving vigorously in the lumen of the tube (Plate 8, fig. 1).

The contents of the hypopharynx of a living infective fly isolated from Batch 2 was examined by inducing the fly to salivate on a cover-glass as described above. On examination of the stained preparations typical blood forms of *T. simia* were seen embedded in the saliva which the fly had ejected on the cover-glass in its efforts to reach the skin (Plate 10, figs. 22 to 25). Another infective fly was taken alive from its glass tube and its proboscis gently squeezed until a minute drop of fluid was observed at its tip, which was then lightly rubbed over a cover-glass. Here again typical blood forms of *T. simia* were found embedded in the salivary secretion (Plate 10, figs. 26 to 29).

In Experiment 1847 it is seen that a positive result is associated with the finding of infected flies in which *T. simia* resembling those found in the blood of infected animals are found in large numbers blocking up the tube of the hypopharynx.

The experiment of tempting the infective fly to feed through a cover-glass demonstrates the fact that when the fly salivates, as it undoubtedly does in puncturing the skin, these blood forms of the parasite are washed out of the hypopharynx with the saliva and are injected with it under the skin of the fly's victim.

Further examination of flies by inducing them to salivate on cover-glasses revealed the fact that sometimes the long, narrow intestinal forms of trypanosomes are ejected in large numbers on to the cover-glass (Plate 10, figs. 1 to 11). There is no doubt, therefore, that an infected fly has the power of regurgitating the contents of its proventriculus and intestines forward into the labial cavity and probably into the blood stream of the bitten animal. It is conceivable that in this way the proboscis first becomes infected by the intestinal forms of trypanosomes, which attach themselves to the inner surface of the labrum and enter the lumen of the hypopharynx, which they invade, however, only as far as the entrance of the two salivary ducts. Here in the chitinous hypopharynx they establish themselves and, bathed in the salivary secretion, finally complete their development into the infective blood form of the parasite.

It is a curious fact that neither the salivary glands nor even the salivary ducts beyond the hypopharynx have ever been found infected with *T. simia*.

It was proved by the Commission in Uganda that the blood forms of *T. gambiense* developing in the salivary tract were the virulent forms of the parasite, and it now seems also proved that the developmental forms of *T. simiae* found in the hypopharynx represent the last and infective stage of development of this species of trypanosome in the "fly."

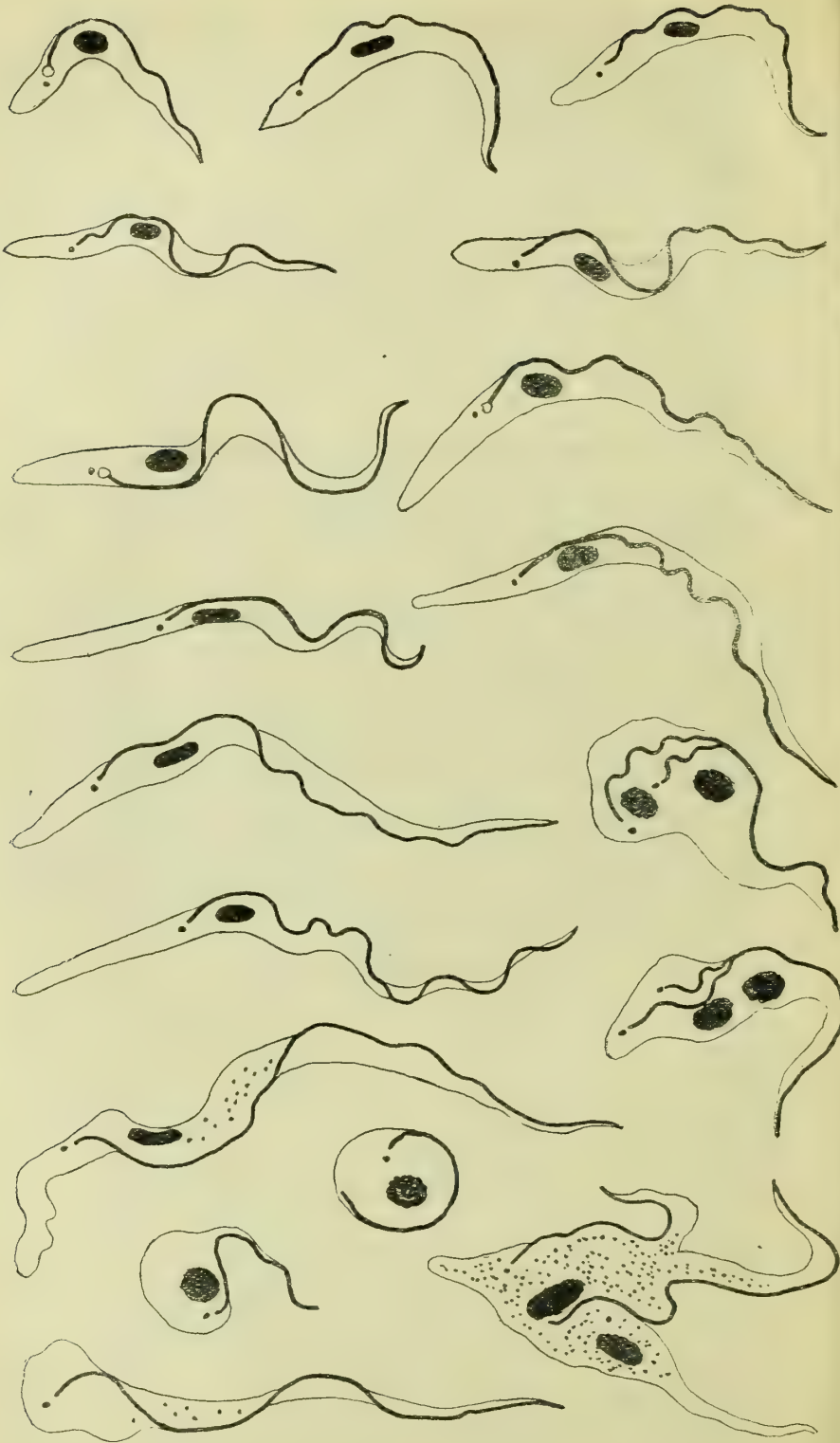
It may be noted here that in the negative Experiment 1477, in which an infected fly was found (Table V.), the labial cavity was infected with the long forms of the parasite attached to the labrum, but most careful search failed to reveal infection of the hypopharynx with blood forms. In this fly the parasite had not attained the final and essential stage of its development—the reversion to the blood type—and so the fly was harmless.

THE TRYPANOSOMES FOUND IN THE ALIMENTARY CANAL.

The intestines of infected flies were generally packed full of trypanosomes from the proventriculus to the mid-gut. Sometimes the infection extended to the hind-gut, but never beyond.

Little need be said in regard to the developmental forms found in the intestines. One curious fact, however, emerges and that is, that it is impossible to differentiate one species of trypanosome from another by the study of these intestinal forms. Whether it is *T. brucei* or *T. gambiense*, *T. pecorum* or *T. simiae*, they present the same appearance. Perhaps on further work some differences may become apparent, but at present no difference has been found to exist. The most numerous forms are long slender, ribbon-like, very active trypanosomes, which in a former paper* were called the normal reproductive type. By comparing Plate 9 with the insets of that paper, the resemblance between the intestinal developmental forms of *T. simiae* and *T. gambiense* will be apparent.

* S.S. Reports XI., p. 34, &c.

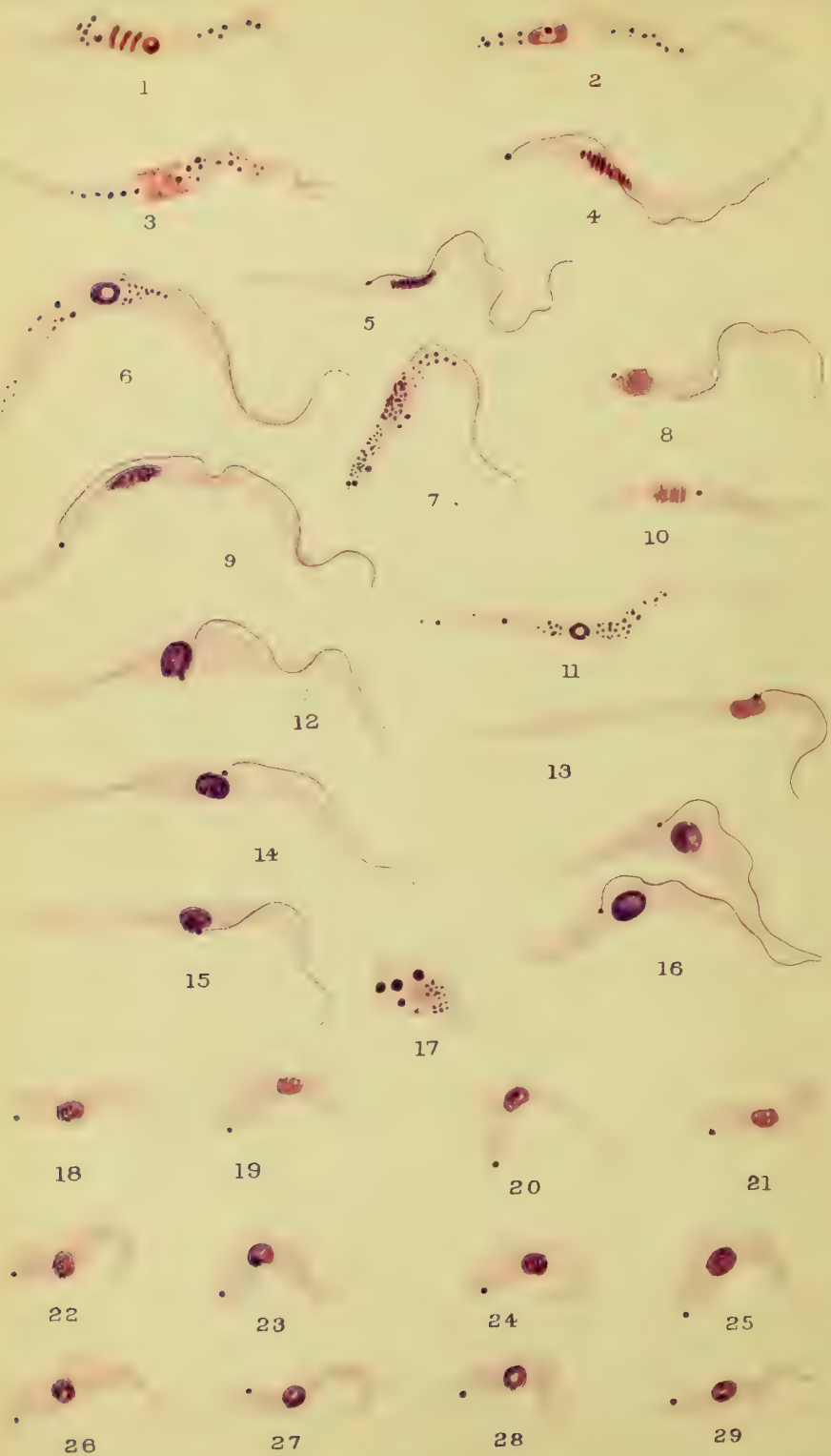


T. Simiæ in gut of fly.

Major Hamerton, del

x 2000.





M E Bruce del

x 2000

Ruth lith et imp

T. sinuæ from proboscis & hypopharynx.

Conclusions.

1. That *T. simiæ* can be transmitted from infected to healthy animals by the tsetse fly *G. morsitans*.

2. That *T. simiæ* multiplies in the intestines and in the labial cavity of the proboscis of the "fly." Here only developmental forms are found, never infective forms.

3. That the *T. simiæ* growing in the intestines of the "fly" has no specific characters by which it can be distinguished from other species of pathogenic trypanosomes found in tsetse flies.

4. That the final stage of the development takes place in the hypopharynx, wherein the infective form of the parasite, similar in shape to the trypanosome found in the blood of infected animals, is produced.

5. That the flies do not become infective until about 20 days after their first infected feed.

DESCRIPTION OF PLATES.

PLATE 8 (p. 136).

Fig. 1.—Appearance of the labial cavity of the proboscis of *Glossina morsitans* with *Trypanosoma simiæ* growing in clusters attached by their flagellar extremities to the inner surface of the labrum. Living and unstained, $\times 500$.

Fig. 2.—Appearance of the hypopharynx in the same fly, showing innumerable small and active *T. simiæ* almost blocking up the lumen of the duct. Living and unstained, $\times 500$.

PLATE 9 (p. 140).

Intestinal developmental forms of *T. simiæ*. These do not differ in appearance from the developmental forms of other species of pathogenic trypanosomes found in the intestinal tract of tsetse flies.

PLATE 10.

Developmental forms of *T. simiæ* from the labial cavity and hypopharynx of *G. morsitans*.

Figs. 1-11.—Trypanosomes ejected by a living *G. morsitans* on attempting to feed through a cover-glass. These are supposed to be intestinal forms pressed up into the proboscis and on to the glass by the muscular contraction of the fly.

Figs. 12-16.—*T. simiæ* growing in the labial cavity of the proboscis of *G. morsitans*.

Fig. 17.—Aberrant form from hypopharynx.

Figs. 18-29.—Blood forms of *T. simiæ* found in the hypopharynx. These form the final stage in the developmental cycle of this species of trypanosome and are the only infective forms. Stained Giemsa, $\times 2,000$.

XIV.—ROYAL SOCIETY'S COMMISSION ON TRY- PANOSOMIASIS IN NYASALAND, BRITISH CEN- TRAL AFRICA. FIRST PRELIMINARY REPORT ON THE BIONOMICS OF *GLOSSINA MORSITANS*.

By Professor ROBERT NEWSTEAD, F.R.S., etc., with the assistance
of Dr. J. B. DAVEY.

(Plates 11-13.)

Owing to the supposed increase of the tsetse-fly, *Glossina morsitans*, in the Nyasaland Protectorate and the menace which it offers, apart from sleeping sickness, to the commercial prosperity of the country, it was deemed desirable that trained entomologists should study seriously the bionomics of this species of tsetse-fly, with the view chiefly of:—

1. Discovering the breeding grounds, as no such study had been previously undertaken, and
2. Devising, if possible, some means for its control, so that the authorities might be in a better position to check the spread of sleeping sickness in the Protectorate and elsewhere in Africa.

Such were the sentiments embodied in a communication addressed to the Colonial Office by Dr. Arthur G. Bagshawe, the Director of the Sleeping Sickness Bureau, under date 15th March, 1911.

Professor Newstead was instructed to take part in the preliminary investigations and left England for this purpose on June 9th, 1911, and was absent for a period of five months. Dr. J. B. Davy, M.O., of the Protectorate, was seconded by the Government as Entomologist to the Commission, and he joined the expedition at Zomba on July 21st.

While this report deals almost exclusively with the bionomics of the tsetse-fly, *Glossina morsitans*, there are certain correlative aspects which are necessarily involved, and it has been thought advisable to introduce these into the body of the work. Matters relating indirectly to the subject of Trypanosomiasis and blood-sucking Arthropods other than tsetse-flies are dealt with in the Appendices,* of which the following are the subject headings:—

1. Big game and other mammals observed in the fly-area of the Upper Shire Valley. (Appendix I.)
2. A list of the birds specifically identified with records of the food found in the crops of various species. (Appendix II.)
3. On the mosquitos and other blood-sucking Arthropods. (Appendix III.)

As our itinerary through the fly-infested country was a short one it will be necessary only to make the briefest reference to it, all the more so as nearly the whole of the country traversed by us was in a very large measure identical with that in which we finally fixed our camp and conducted our investigations. Our

* These Appendices are not printed.

main object in travelling through the fly-area was chiefly to discover a locality in which there was an abundance of tsetse-flies and also a liberal supply of water, necessary both for ourselves and the natives employed by the Government to assist us in erecting a mudhouse with apartments for sleeping and a laboratory combined.

In the first instance it had been suggested by His Excellency the Governor, Sir William Manning, K.C.M.G., that the district lying between the Zomba range and the Shire River, extending from the Zomba-Liwonde road to the Majusi stream, west of the Namadidi estate, might form a suitable place for our investigation—firstly because *Glossina morsitans* had been found there in some numbers, and secondly that it was within comparatively short range of the seat of Government, being about 23 miles distant from Zomba. Through the kindness of His Excellency the Governor, who very willingly conveyed one of us* by motor car to the locality, an examination of a portion of the tsetse-fly belt was made on July 18th. *Glossina morsitans* was seen there in some numbers, but as they were not considered sufficiently numerous to afford us unlimited opportunities for research, this site was abandoned, and a few days later we proceeded to Liwonde.

As the fly-area had been found to be more or less continuous between Liwonde and Lake Malombe we decided to traverse those portions of it in which, as he had been informed, the largest number of tsetse-flies had been found by Europeans. At first we explored the eastern side of the Shire, traversing the country up to the foot of the hills some seven miles distant. We subsequently crossed and recrossed the Shire, making detours on both sides of the river and finally reaching as far north as the hills beyond Changani, near the main Fort Johnston-Liwonde road. Having carefully noted the relative abundance of the tsetse-flies in the various localities through which we had travelled we came to the decision that the most suitable place for us to conduct our investigations was a district lying about four miles south of the Lake Malombe. Hereabouts the fly-area extended to within a mile of the river, so that by pitching our camp near to its banks we were within short range of the two most essential things—tsetse-flies and water. This station was occupied until the beginning of November, when the site was abandoned, and the Permanent Entomologist, Dr. J. B. Davey, proceeded shortly afterwards to the main camp at Dowa to continue his researches in conjunction with the other members of the Commission under the direction of Sir David Bruce, F.R.S. The information embodied in this report deals therefore with the observations made during our stay in the Upper Shire, covering in all the period from July 18th to November 2nd, and although we met with a fair amount of success we offer the results as a preliminary to further investigations, since it must be perfectly obvious that no very definite results can follow an enquiry of so short a duration. Many more months of patient labour must necessarily follow before the many and various problems connected with the bionomics of *Glossina morsitans* can be made manifest.

* Professor Newstead.

The physical features of the country in which we fixed our camp may be taken as typical of the whole of the Shire valley lying between Liwonde and Fort Johnston. It consists of a broad alluvial plain through which the river flows. On the western side the low-lying "dambo" or savannah is on the whole more extensive than it is eastward of the river, and consists very largely of a particularly tenacious clay which, during the dry season, is rendered as hard as a sun-dried brick; but there is evidence of much of this kind of country becoming more or less water-logged during the rains. In places the slightly higher ground extends almost to the fringe of the river, and consequently the nature of the vegetation is somewhat different. The elevation above sea-level is between 1,200 and 1,650 feet: the former being the figures for Liwonde, the latter those for Fort Johnston.

The total rainfall for Liwonde* during the year 1908 was 27·41 inches, while the total rainfall for the whole of the "wet season" (November, 1908, to April, 1909, inclusive) is recorded from the same station as 29·44 inches. This total is considerably lower than that given for the majority of the other meteorological stations in the Protectorate, including also the record from Fort Johnston, though the latter lies but some 45 miles north of Liwonde. The dry season commences in May and is continued until the end of September. In the year 1908 14 inch of rainfall was recorded at Liwonde during the month of May, and ·02 inch in October. Of the intervening months the record given is nil.

As to the temperature and humidity of this part of the country we can give no reliable information as through accident and delay the more important instruments were not available, and we were unfortunately obliged to fall back upon an ordinary thermometer which, though useful in a small measure was altogether inadequate for our investigations, so that in the absence of other sources of information we do not feel justified in offering any further remarks on this subject.

Types of Vegetation.

The flora may be briefly considered under three geographical divisions; though in the absence of suitable material during the dry season, we cannot do more than refer, very briefly, to a few plants and trees which were found to predominate. These areas are:—

1. The river and its borders.
2. The dambo.
3. The forest or fly-area.

In the first of these areas the vegetation is decidedly variable. In places where the river banks are low and partly or wholly submerged during the rains, the great plume-bearing *Phragmites* reed forms a distinctive feature of the landscape; but where the

* Handbook of Nyasaland (Second Edn. 1910), p. 222.



FIG. 1.



FIG. 2.

To face page 145.]

banks are elevated they are clothed with various forest trees, many of which attain large dimensions; and beneath them there is often a dense and almost impenetrable undergrowth of scrub. Palms too may generally be seen in such places, though these graceful trees are usually confined to the semi-open spaces, especially so towards the fringe of this taller vegetation. In many places two-thirds of the river is rendered impassable by a dense floating platform of aquatic plants which forms a retreat for innumerable crocodiles and a cover also for the hippopotamus. This so-called "sudd" consists very largely of grasses and in places extensive colonies of papyrus; while the fringe is composed chiefly of the beautiful but obnoxious "water caltrops" (*Trapa bispinosa*); and the floating "cabbage" or "duckweed" (*Pistia stratiotes*).

The second of these areas is composed largely of open country with extensive tracts of thorny scrub, consisting chiefly of flat-topped and other acacias, the huge cactoid *Euphorbia*, a species of *Albizzia*, flowering profusely in September and forming quite an attractive feature of the country. Palms (*Borassus flabellifer*) occur at intervals, but they are by no means plentiful; while the "sausage tree" (*Kigelia* sp.) is also present, though sparingly distributed. Baobabs are almost everywhere a characteristic feature, but are most plentiful a few miles north of Liwonde. The *Strophanthus nicholsoni* is, in places, quite common, and in the open country is distinctly shrub-like in its habits; though its younger branches are always struggling upwards entwining each other in the vain effort to secure support; its quaint bicornate fruits, and its nodose branches are also strikingly characteristic and cannot fail to attract the attention of the traveller. *Combretum microphyllum* is an immense climber, reaching to the crowns of some of the taller trees and putting forth, in September, such a profusion of scarlet flowers as to almost obliterate the leaves of its host. It is, however, much more common near the river and thrives best apparently in moist situations.

As to the nature of the undergrowth one can say very little, as nearly all the plants were dead and dry, or completely destroyed by the grass fires. The Macuna bean or "cow-itch" is sometimes found in such abundance as to completely bar ones progress, especially so where there is a tangled vegetation, but it is equally well supported by the tall "elephant grass" as it is by the branches of trees and shrubs. It is a most atrocious pest and the pain produced by the urticating hairs which clothe its fruiting pods is almost beyond endurance. Various grasses are the predominating plants in the open, but here and there may be found enormous tracts of *Asparagus*, and the bulbs of other liliaceous plants were sometimes found rotting up by wart hogs. In old clearings and native gardens *Adenium multiflorum* is a great pest, as also were the seeds of a species of *Compositæ*.

As to the forest proper the trees comprising it are for the most part distinctly "short" in character, in fact the whole of it may be described as typically "low forest" (figs. 1-2, plate 11), and in this respect it is more or less characteristic of that of the whole of the country. The relative density of the forest varies considerably:

in some places the trees are fairly close to each other, but, even so, small open areas are frequent, and much of the country is more or less park-like and generally flat, though slightly undulating in places. The tree which preponderates in this region is the Sanya or Iron-wood tree (*Copaifera mopanie*, Kirk); in fact it is so abundant that those portions of the forest in which it abounds are referred to as "Sanya country" both by natives and Europeans. In the dry season it supports a scanty foliage and in the distance bears a striking resemblance to a small and rather lanky elm. The leaves though hard and rather dry are eaten extensively by the Impala antelope (*Apyceros melampus*) and more sparingly also by other antelopes.

Beneath such trees the land is generally clothed with short grass which adds considerably to the park-like appearance of the country. It must be distinctly understood, however, that there is not an unbroken line of sanya trees. Many are the changes in the character of the flora even in relatively small areas. In places there may be a rather dense undergrowth of shrubs or scrub with an almost total absence of grass. Isolated clumps of trees occupying the low mounds of deserted "anthills" (Termites) are quite a feature in some parts of the country, especially where the forest merges into the semi-dambo areas; such clumps, especially the older ones, often have a shade-giving undergrowth of straggling and non-deciduous shrubs (fig. 1), whilst the surrounding land may be clothed with the dwarfer kind of grass. Baobabs too are often seen within the forest belt; ebony (*Diospyrus* sp.) is very common; but *Erythrina* sp. less so. The giant creeper (? *Landolphia* sp.) (fig. 3, pl. 12) with its soft, cork-like tissues and serpentine folds climbs to the tops of some of the taller trees; sending down long lianes and fruiting branches, and in August its beautiful pappus-bearing seed lies scattered over the land like giant "thistle-down." It is one of the most grotesque as well as one of the most characteristic plants of the forest. In more open country one finds a leguminous tree in some abundance, its enormously long pendulous fruiting pods hanging from its somewhat slender branches in great profusion. Antelopes eat portions of the pod, and grey hornbills and squirrels extract the small beans. There is some doubt as to its botanical name, but it is possibly a representative of the genus *Parkia*. A beautiful yellow-flowering Cassia (? *G. goratensis*) grows in similar bush-like country, but is not very common. In such country and amongst such surroundings the Impala antelope makes its home and the common tsetse (*Glossina morsitans*) propagates its species. Three things are therefore apparently correlative: The Sanya tree, with shady undergrowth, the Impala antelope and *Glossina morsitans*; or in other words "Sanya country" and "Fly country" are one and the same and such also is the favourite resort of one of the commonest of the vertebrata in the Upper Shire. This, however, is but a local picture of a portion of the fly area in Nyasaland* as seen during the winter; when the tropical sun pours through the almost leafless trees rendering the

* It is well known that *Glossina morsitans* occurs in other parts of Nyasaland where sanya trees (*Copaifera mopane*) are absent.



FIG. 3.



tenacious soil indurate and robbing it of all moisture. There is practically no humus, certainly no sand, and there is little else but sunbaked clay until the rains commence in October when the land becomes plastic and fertile.

The Vertebrate Fauna of Lake Malombe District.

Broadly speaking the fauna of this area may, we think, be taken as fairly representative of that of the whole of the Upper Shire Valley lying north of Liwonde, so far at least as it is represented during the dry season from July to October inclusive. There was little evidence of migration among the birds; and to what extent the dry season effects the presence of the larger game we are not prepared to say, but it is generally held by those who have resided for long periods in the Protectorate that a larger proportion of game is found in the vicinity of the river during the dry season than at other periods when the forest pools and the tributaries of the Shire are well stocked with water. Some of the antelopes, especially Nswala or Impala (*Æpyceros melampus*) may, however, be considered as "residents," and the same may be said also in regard to the wart hog (*Phacochoerus aethiopicus*), and a small squirrel (*Funisciurus cepapi*), which is common and generally distributed over the whole area. The Impala antelopes were so abundant and also so constantly present in the fly-infested areas that it was only reasonable to infer that these animals, at least so far as this region is concerned, must necessarily supply a very large proportion of the food necessary for the life and propagation of the *Glossina*. On two occasions we were able to see, by the aid of strong binoculars, that these animals were being constantly irritated by flies, probably tsetse, as no other biting flies were either seen or captured in any part of the forest where this antelope occurred. Large packs of the baboon (*Papio babium*) were seen, so that if tsetse flies have any predilection for these animals they had occasional opportunities for asserting it. Of the reptiles few were seen actually in the fly areas, though representatives of the Squamata such as *Varanus? albigularis*, and the smaller lizards were common on the banks of the river and its environs. Of the Ophidia, one of the burrowing snakes (*Typhlops* sp.) was the commonest and most frequently observed; the python (*P. sebae*) and the puff adder (*Bitis arietans*) were also seen in the immediate neighbourhood of the Shire, but quite outside the fly area; while the interesting and highly protective tree-frog (*Chiromantis xerampelina*, Petus) seemed generally distributed over the whole area in suitable localities, one of its favourite retreats being the deserted nests of certain weaver birds (*Ploceipasser* sp.).

Birds were common everywhere, and altogether 78 species were specifically identified; of these by far the larger proportion were either strictly insectivorous or partially so, and therefore one may safely assume that they would be the most unlikely to be attacked by tsetse flies; indeed we have evidence to prove that at least two species (*Melittophagus meridionalis* and *Dicrurus afer*) prey upon *Glossina morsitans*; so that it is highly improbable that any of the insectivorous kinds are likely to form the natural food supply

of blood-sucking insects. On the other hand there are a certain number of birds which, judging by their habits, may occasionally fall a prey to the attacks of tsetse flies; such for instance as the owls (*Scops capensis*, *Strix flammea** var., &c.), especially the pretty little scops, as it sits helplessly upon the bare branches of the trees during the hours of daylight and allows itself to be captured with the greatest ease and without offering any marked attempt to escape. There are also innumerable parrots (*Paeocephalus fuscicapillus* and *Agapornis lilliana*) which occur in large flocks or small companies throughout the forest areas. Doves of three kinds were extremely abundant and at dawn and sunset frequented the pools in the forest in enormous numbers, coming and going for an hour or more at each period of the day respectively, in one almost continuous stream. Turacos, especially *Schizornis concolor*, were also common and generally distributed. The common guinea fowl (*Numida cornuta*) and one of the francolins (*Pternistes nudicollis*) occurred everywhere, but the former was by far the more numerous species, indeed it was so abundant that it was considered desirable to secure living examples in order that we might put our theory regarding the natural food supply of *Glossina morsitans* to the test. Two birds were therefore taken into the fly area and secured in different places known to us as the favourite resort of the tsetse. They were carefully watched, and although the fly was in evidence it made no attempts to attack the birds; indeed it seemed to avoid them rather than otherwise. Of all the birds frequenting the forest and dambo very few offer such vulnerable parts for attack as the guinea fowl, the head and neck being destitute of feathers could readily be pierced by the proboscis of *Glossina*. On the other hand the thick covering of feathers present in the majority of birds would, we feel certain, render them practically invulnerable to tsetse-flies, and for this reason we think it highly improbable that many of them would form an important source of the natural food-supply of these insects. No microscopical examinations of the food contents of the alimentary canal of *Glossina morsitans* were made, as this phase of the work was considered outside the pale of our investigations. But Sir David Bruce† has shown that birds are not the important source of food that one might imagine them to be, seeing that tsetse-flies feed so readily upon the domestic fowl when in captivity.

Further information regarding the mammalian and avian fauna is given in the Appendices Nos. I. and II. respectively.

Bionomics of Glossina morsitans.

Relative abundance.—Almost at the very commencement of our work we found that persons wearing Khaki clothing were more attractive to tsetse-flies than either the semi-nude native or others of them who were clothed with variously colored garments; we decided therefore to use Khaki as an index to the prevalence of the flies during our peregrinations. And after many weeks

† Proc. Roy. Soc., 1910, B, 558, pp. 490-497. (S.S. Reports, XI, p. 112.)

of observation we found that the mean maximum number of flies attacking or resting upon a Khaki jacket, worn by one of us, in any portion of the fly infested area was 11. Only on very rare occasions did we find a larger number present at any given time, and even so the maximum never exceeded 14. Moreover we often travelled for long distances without seeing more than three or four individuals in any given spot, and in many places they attacked us singly. They were never seen to swarm as they have been observed to do in other parts of Africa; but we particularly noted that we got a higher index when we were on the march than at times when we were stationary.

Only on very rare occasions were tsetse-flies observed in semi-dambo country or thorny scrub, and never so in the open dambo or in any portions of the country immediately bordering the Shire river. The natural haunt of *Glossina morsitans* is undoubtedly the low forest country, the nature of which has already been discussed (pp. 145-6). We noted, however, that tsetse repeatedly followed us into the semi-dambo country, and on several occasions individuals accompanied us right into our camp when we approached it through the thin scrub which intervened between us and the tsetse area; they even attempted to bite us on two or three occasions while we were in our tents or dining hut, but tsetse-flies were never seen either in or near our camp under any other circumstances. We are confident therefore that the short distance between the river and the fly area, though protected and shaded to some small degree by thin scrub for the whole of the distance, was never to our knowledge voluntarily traversed by tsetse.

Having observed that *Glossina morsitans* never occurred near the Shire river, although in places the nature of the country seemed very suitable, it was decided to liberate a number of marked flies in such localities, with the view of ascertaining whether they would remain there or return to the tsetse-country, about a mile distant. Unfortunately the number of flies employed in the experiments were much too few to enable us to obtain satisfactory results, but they were all that could be secured at the time. Two sites were selected; the exact nature of which was as follows:—

Site No. 1. Soil distinctly arenaceous, trees tall and plentiful, the majority more or less leafless, but some were evergreen, and all of them surrounded by dense bush with small clearings intervening. Sanya (*Copaifera mopane*) absent. In this place crocodiles came to bask and lay their eggs. It was also the haunt of the bush-buck (*Tragelaphus scriptus*); Nswala or Impala passed through to drink; guinea-fowl frequented it also for the same purpose and numbers of them also came to roost in the trees. The wart-hog and the baboon were sometimes seen here, and a mon-goose was not uncommon. This belt of trees and scrub varied in width from 50 to 100 yards, about; and was continuous along the bank of the river for a distance of approximately one mile. A small gully or stream-bed arising in the tsetse or sanya country passed through the belt of trees to the river. This gully had a continuous fringe of trees, most of them small,



FIG. 4.



FIG. 5.

To face page 151.]

quarters of an inch below the surface and was lying so that it almost touched the bark of the tree. The soil was decidedly hard, so hard in fact that it was not easily broken into small fragments with a trowel. The tree in question formed part of an isolated group (fig. 4, pl. 12), consisting chiefly of two other sawya trees, two or three ebonys (*Diospyrus sp.*) and several tall shrubs the leaves of which, for the most part at least, were green and afforded together with the dwarfer undergrowth an ample amount of shade. This isolated group of trees and plants was growing on an old deserted termites' nest which had been weathered down so that its original height had been considerably reduced; but the whole of the mound was sufficiently raised above the flat surrounding country to stand out in marked contrast with the rest of the "bush." One of us had previously noticed that *Glossina morsitans* was generally present in such isolated clumps of trees and scrub of which there were similar ones scattered freely throughout the tsetse country. This observation led us, moreover to believe that such places might be selected by this tsetse-fly for the purposes of depositing its larvae, all the more so seeing that these small hillocks would not get water-logged during the rainy season, and moreover because we had also found that the vegetation in such places had almost invariably escaped the grass fires. The animals found in association with the pupa of *Glossina* included a small colony of ants, a few inches away; pupae of noctuid moths and many examples of a large millipede, each one occupying a large and smoothly lined earthen cell; a small and highly protective beetle; and the pupae of various muscid flies. Date 12. VIII. 11.

2. The second pupa of *G. morsitans* was found on the same day; and, like the first, was unfortunately empty. In this instance it was lying at the foot of a large tree (apparently a species of *Erythrina*) or rather between the tree trunk and a very small termites' nest, being buried in the earth which had accumulated between the tree and the nest to a depth of about four inches from the surface.

3. The third pupa, a living one, was found just below the surface in a small quantity of earth which had accumulated in the cavity of a large ebony tree on a level with the surrounding surface-soil. Here the soil though rather hard was of a much more friable nature than that in which the pupae were found on the previous occasions. The tree in question formed one of a small isolated group (fig. 5, pl. 13) all of them occupying an old termites' nest. The opening to the cavity in the tree faced almost due south and measured about three inches wide. The animals found in association with this pupa were of a similar kind to those found elsewhere, the millipede being particularly numerous. Date 17. VIII. 11. Distance from river one mile, about.

4. The fourth and also perfect pupa was found in loose earth which had accumulated in a deep recess formed by the buttressed roots of a baobab tree (*Adansonia digitata*), facing and exposed to

the south-west. Sunlight would not, however, be able to enter the recess until the late afternoon, if at all. There were some small leafless bushes (*Strophanthus nicholsoni*, etc.) on one side of the baobab, but these could scarcely be said to form a distinct group such as has been noted in the other habitats. The baobab was situated in rather open "sanya country" about two miles inland from the river. Date 16th October, 1911.*

From these observations we are of opinion that the breeding grounds of *Glossina morsitans* in this region are thinly scattered over the whole of the tsetse country, and it is quite evident that the pupae, whether living or dead, do not occur in large numbers in any given spot. In localities where the flies are much more numerous than they are in the Upper Shire Valley, larger numbers of pupae would probably exist in similar habitats.†

Breeding Experiments (Female).—The method adopted for breeding tsetse-flies was similar to that employed by other investigators. In the majority of instances, however, two males and one female, all freshly caught, were placed together in one glass vessel, though in a few instances the numerical order of the sexes was reversed. The object in not mixing the females was to enable us to follow as accurately as possible, the periods between the acts of parturition and other phases in the metamorphoses and bionomics of this insect. Native fowls were invariably employed in the feeding experiments and although these animals were offered to the flies daily, it will be seen on referring to the table (p. 153-4) that the number of meals taken by 41 females varied to a marked degree; but the average duration of time between each meal was 2·56 days. In many instances a meal of blood was taken four days before parturition, the minimum number of days being three, the maximum five days; while in several cases the females fed a few hours after producing a larva. It will be seen, furthermore, that the period which elapsed between the date of capture and that of the production of the larva varied to a very great extent, the minimum being two days, the maximum 29 days. Apparently therefore the breeding season is continuous through the dry season and this view is supported by the fact that a living pupa was found in a state of nature; but there is some evidence (greater abundance of females immediately after heavy rains) that breeding may be more extensive at the commencement of the rains and possibly also at the end of the wet season than during the dry months. As to the period between the production of the individual larvae we can unfortunately say very little, as in one instance only did a female survive after the first larva had been produced, and in this case, unfortunately, the larva was aborted. In two instances the pupal period lasted for 26 days and in one instance 24 days.

* It may be noteworthy to add that this pupa was found three days after very heavy rains.

† Mr. Rupert W. Jack, Government Entomologist, has recently found this to be the case in Lomagund, Rhodesia. Bull. No. 100. Dept. Agric., Salisbury, Rhod. Oct., 1911.

Table No. 1, showing results of breeding experiments with captured females. Date, September 11th to November 3rd, 1911.

Female lived —days.	Number of days on which food was taken.	Produced larva after capture —day.	Intervals between acts of parturition —days.	Pupal period —days.	Remarks.
14	8	—	—	—	
9	4	—	—	—	
30	12	20th, 28th	8	24	Sex. The second larva was aborted and did not pupate.
13	8	—	—	—	
3	—	—	—	—	Copulation observed on first day.
3	—	—	—	—	
16	4	—	—	—	
15	9	12th	—	—	Survived three days after parturition. Larva preserved.
11	5	6th	—	—	Larva aborted.
10	3	—	—	—	} Isolated from males to ascertain if pregnant.
11	4	—	—	—	
7	5	—	—	—	On 6th day pair in coitu and not fed.
37	20	29th	—	—	Larva pupated, but pupa shrivelled subsequently.
7	1	—	—	—	
8	5	—	—	—	Experiment discontinued through an accident.
29	8	10th	—	—	Had not produced fly on 30th day and was preserved.
3	1	—	—	—	
11	?	7th	—	—	{ Both females were kept in the same jar. Pupa of one preserved. The other did not produce imago.
13	?	9th	—	—	
11	7	—	—	—	Female escaped on the 11th day.
2	2	—	—	—	
10	4	—	—	—	Female escaped on 10th day.
28	6	—	—	—	
21	10	18th	—	26	The imago was a female. The parent female escaped on the 21st day.
16	7	15th	—	—	Pupa presented when camp was abandoned.
5	3	—	—	—	Female escaped on 5th day.
23	9	9th	—	—	Pupa defective.
1	1	—	—	—	Possibly injured by capture.
10	7	—	—	—	
20	8	13th	—	—	Failed to pupate.
17	3	—	—	—	
12	4	—	—	—	
9	5	—	—	—	

Table No. 1.—*continued.*

Female lived—days.	Number of days on which food was taken.	Produced larva after capture—day.	Intervals between acts of parturition—days.	Pupal period—days.	Remarks.
10	—*	9th	—	—	Larva aborted apparently.
6	2				Female escaped.
21	8	20th	—	—	All destroyed by ants.
18	9	11th	—	—	Breeding jar broken during night (?) by rats.
24	7	18th	—	—	Sex ♀ Parent escaped.
3	1	2nd	—	26	Experiment discontinued.
30	16	23rd	—	—	Female died 5th day after parturition.
26	8	21st	—	—	

Feeding.—In feeding the abdomen is not always filled to repletion, but when fully distended it becomes almost globose. Ventrally it is at first of dull reddish-crimson colour and at this stage the white, branched, tracheal tubes stand out in marked contrast to the other organs. Almost immediately after feeding a clear fluid is freely passed during the first half hour or so which rapidly evaporates and leaves a white granular deposit; the body at the same time gradually loses its rotundity and within an hour in some individuals, the form of the abdomen becomes semi-normal in size; the blood showing through the second abdominal segment as a dull crimson suffused area, but signs of red blood pigment may be observable several days after feeding. Between the second and twelfth hour after feeding defecation takes place frequently, the faeces varying in colour from dark brown to blackish. The actual period involved in feeding varies considerably, but a hungry fly will sometimes fill itself to repletion in half a minute though in such instances the insect may have previously pierced the skin without drawing blood. After a fowl has been fed upon tsetse-flies for a period of two or three weeks it becomes anaemic, when the flies will refuse to feed upon it. When feeding the palpi are slightly raised but so far as one could see remained closely adpressed. A freshly caught male tsetse will, as a rule, bite directly it is offered food; on the other hand we found that females were often slow in feeding and the mortality amongst freshly caught examples was considerably greater than in the males.

Copulation frequently occurred immediately the sexes were placed in the breeding-jars; and the act of coition often lasted for a considerable time. One male was seen to copulate with a dead female and remained in coitu for a considerable time; the male was a freshly caught one, the female had been in captivity for a few days.

Often when liberating freshly caught flies, especially males, they would repeatedly return, making vigorous attacks upon us,

* Figures in the original are obliterated.

sometimes buzzing loudly and viciously. Twice we were bitten by tsetse while transferring them from one vessel to another, and twice also when we were searching for pupae in shady places in the forest. In the latter instances the flies crawled under the wrist while the hand was resting flat upon the grass, and the presence of the fly was not noticed until its proboscis had pierced the skin.

Period of Activity.—*Glossina morsitans* seems as active and as prevalent in the afternoon as in the morning; the period of chief activity being, approximately, between the hours of 10 a.m. and 4 p.m., but there was generally a diminution of tsetses on dull sunless afternoons, more especially so when there was a marked drop in the temperature. Occasionally however they were seen both early and late. Thus for example on August 8th at 6.20 a.m. immediately after sunrise, a specimen alighted upon Khaki coloured garments worn by one of us; there was scarcely any wind at the time and its behaviour was precisely the same as that of others observed during the heat of the day. Again during the first week in August one of us was bitten, while standing at the entrance to the tent at 6.10 p.m., by a tsetse which had evidently followed us from the "bush." It was dark at the time. Flies which were kept in the breeding jars were sensitive of the slightest disturbance on moonlight nights, and specimens which were liberated under such conditions took flight immediately appearing as active as during the day.

Although a number of antelopes were killed in the tsetse area on two occasions only was *Glossina morsitans* attracted by the dead animals (Impala) and then only one fly alighted on each of the two carcasses respectively.

Position of the wings when at rest.—One of the most marked characteristics of the genus *Glossina* is the fact that the wings when at rest, in life, are said to be carried so that they completely overlap like the blades of a pair of scissors. While this habit may hold good for the majority of species it most certainly does not apply to *Glossina morsitans* when in captivity at least, as it carried its wings, *when at rest*, so that the tips were slightly divided. This habit is clearly shown in the accompanying photographs taken from life.

Colour preference.—Reference has been made to the fact that *Glossina morsitans* was seen to be much more readily attracted by khaki-coloured garments than those of any other colour or the naked skin of the natives. During the month of October one of us made a series of observations on ten different days to test the colour preference of this tsetse-fly. For this purpose natives were provided with vests of the following colours: (1) white (clean), (2) bright canary-yellow, (3) red, (4) khaki, (5) dark blue; the bare back of a native being used as a control. The results were that khaki headed the list for attractiveness and in a diminishing sequence red, blue, bare back of a native, and white; no flies were seen upon the yellow garment. Dirty white vests were subsequently found to be much more attractive than clean ones. Furthermore the buff-green canvas such as used for machilla coverings, bags, tents, &c., was found to be quite as attractive as the khaki.

Larvae of Glossina morsitans. — Form somewhat changeable, but when at rest it is subcylindrical, being very slightly flattened dorso-ventrally; integument shiny on the middle of the segments and faintly shagreened. Segmentation well pronounced on the anterior half of the body, but generally less so posteriorly; head with two minute black and rather widely separated appendages with three minute blackish spots arranged in an equilateral triangle near them. There is also a narrow dark and rather indefinite line extending posteriorly from between them. Colour creamy white; tumid lips intensely black with the rugosities slightly polished. Length 5 mm., greatest width of thoracic segments 2 mm., width of abdominal segment immediately preceding the "tumid lips" 1.5 mm. It should be noted that the larva from which this description was made, though kept for a period of 24 hours did not change, and as it seemed to get less active it was killed. The probabilities are, therefore, that it was slightly immature though it appeared perfectly healthy when it was first found in the breeding jar.

The movements of the larva may be described as of three kinds. (1) a peristaltic wave of constriction passing slowly forwards from the tumid lips; (2) a wagging movement of the anterior extremity; and (3) an alternate lengthening and shortening of the whole of the body. In nearly all the cases under observation the larvae were extruded during the night or early morning.

Natural enemies.—Of the many birds which frequented the tsetse-fly area and its immediate neighbourhood, post-mortem examinations of 36 different species were made in order to ascertain to what extent, if any, they fed upon *Glossina morsitans*. Out of the total of 61 birds examined, only two were found to contain the remains of tsetse-flies, though the stomach of a third was found partly filled with another sanguivorous muscid belonging to the genus *Stomoxys*. It would seem, therefore, that tsetse-flies do not enter very largely into the dietary of insectivorous birds, but as such birds were everywhere in evidence they must at least be considered of some potential importance and may hereafter be found to feed upon tsetse more extensively than these records would lead us to believe. Those which had fed upon *Glossina morsitans* were the common black drongo of the country, *Dicrurus afer*, and the small black and yellow throated bee-eater, *Melittophagus meridionalis*. The other birds which are thought most likely to prey upon tsetses are the crow-shrikes (*Prionops* and *Sigmodus*), the grey babbler (*Crateropus*), and the roller (*Coracias caudatus*), all the more so seeing that such representatives of the avian fauna were always in evidence throughout the fly infested areas. Nearly all of these could also be seen congregated together in association with grey hornbills (*Lophoceros* spp.) close up to the grass-fires pouncing down upon the fugitive insects which were forcibly driven into the open; it is highly probable, therefore, that tsetse-flies may be taken at such times as well as under other conditions.

No Hymenopterous insects such as *Bembex* or any member of the Spegidae were seen. The only nests of a predaceous wasp

which were discoverable were those of the pretty little *Sceliphron spinifer* which had stored its cells with rather large spiders.

On searching the webs of a spider's-nest which was extremely abundant throughout the whole of the Shire valley, the remains of one example of *Glossina* was found. Unfortunately the specimens of the arachnid which were collected for the purpose of identification got so damaged in transit that they were rendered quite useless.

Bird-lime for trapping tsetse-flies.—The natives of Nyasaland manufacture a highly viscous bird-lime from *Loranthus kalarhariensis*, Schinz. As we were wishful to test its applicability for capturing tsetse-flies we gave instructions for a small quantity to be made. The results were disappointing, as the material proved much too elastic to be of any real value for trapping small insects. Some tsetsefluges were also experimented with, but the results were so unsatisfactory that they are scarcely worth recording.

May, 1912.



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INTRODUCTION.

This, the first Commission sent out to Nyasaland under the direction of the Royal Society, was formed at the suggestion of Mr. Harcourt, Secretary of State for the Colonies. The terms of reference were to enquire into the relation of the African fauna to the maintenance and spread of trypanosome diseases of man and domestic animals. The period of three years was laid down as the probable duration of the enquiry and the cost was not to exceed £5,000 a year.

The members of the Commission were: *Director*, Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; *Members*, Majors David Harvey and A. E. Hamerton, D.S.O., R.A.M.C., Dr. J. B. Davey, Nyasaland Medical Staff, and Lady Bruce, R.R.C.; *Laboratory Attendant*, Staff-Sergeant A. Gibbons, R.A.M.C.; *Clerk*, Mr. James Wilson.

Dr. Davey resigned from the Commission in October, 1912, and Major Harvey also resigned after serving some twenty months and left for England on the 16th September, 1913. The latter's place was filled by Captain D. P. Watson, R.A.M.C., who left England in September, 1913, and arrived at Kasu on the 2nd November.

Major Hamerton went to Nyasaland in advance of the Commission to assist the local authorities in selecting a suitable site for the camp, and to make preliminary arrangements. He left England on the 14th July, 1911, and arrived at Zomba, the official capital of Nyasaland, on the 1st September. With the assistance of Mr. W. Kirby Green, the Magistrate of the Dowa District, and Mr. T. I. Binnie, the Director of the Public Works Department, he selected a site for the camp. Kasu, the locality selected, is a hill near the edge of the Angoniland Plateau, in view of Lake Nyasa, and overlooking the tsetse fly infested plains lying between the Lake-shore and the hills (see map opposite). It is the centre of a district where there is ample material in the way of tsetse fly, game, stock, and cases of sleeping sickness, for the investigation of the Commission.

The Director and the remaining members of the Commission left England at the beginning of November, 1911, and arrived at Kasu Camp on the 12th January, 1912. Here they met Major Hamerton and found the laboratory, office, and Director's house completed, so that work was begun at once. The laboratory and houses were built of native-made bricks, the inner walls of sun-dried bricks, the outer or those exposed to the weather of fired bricks. The roofs were thatched with grass and the houses proved cool and comfortable.

Kasu Camp (latitude 30° 40' S., longitude 34° 12' E.) is situated about 30 miles to the west of Lake Nyasa and about 3,500 feet above sea-level. It is placed on a flat ridge about 500 feet below

the summit of Kasu Hill and commands a fine view of the surrounding country. The nearest "Boma" or magistracy is Dowa, 18 miles to the west. In the immediate vicinity is the Mvera Mission station, belonging to the Dutch Reformed Church (see Map facing p. 1).

The climate at Kasu is temperate and pleasant, the thermometer in the shade rarely rising to 80° F. in summer, or falling below 60° F. in winter. The year is divided into a well-marked dry season, from April to November, and a rainy season, from December to March. Thermometer readings during 1912, 1913, and 1914 are given in Appendix I.

The camp consisted of a laboratory capable of seating six workers, with an adjoining room for sterilising and washing-up purposes. Two large tanks supplied the laboratory with water by gravitation. Near at hand was the office, consisting of two rooms for the Director and Clerk. Four dwelling houses, animal houses, stores, and destructor were also provided. The water supply for drinking purposes was obtained from a spring situated about half a mile to the east of the camp. This was walled in and provided with a lid and padlock. It ran all the year round and provided excellent drinking water. The ordinary supply for the camp was obtained from a small valley running on the south side. Here a well was dug near the small stream and a pump fitted.

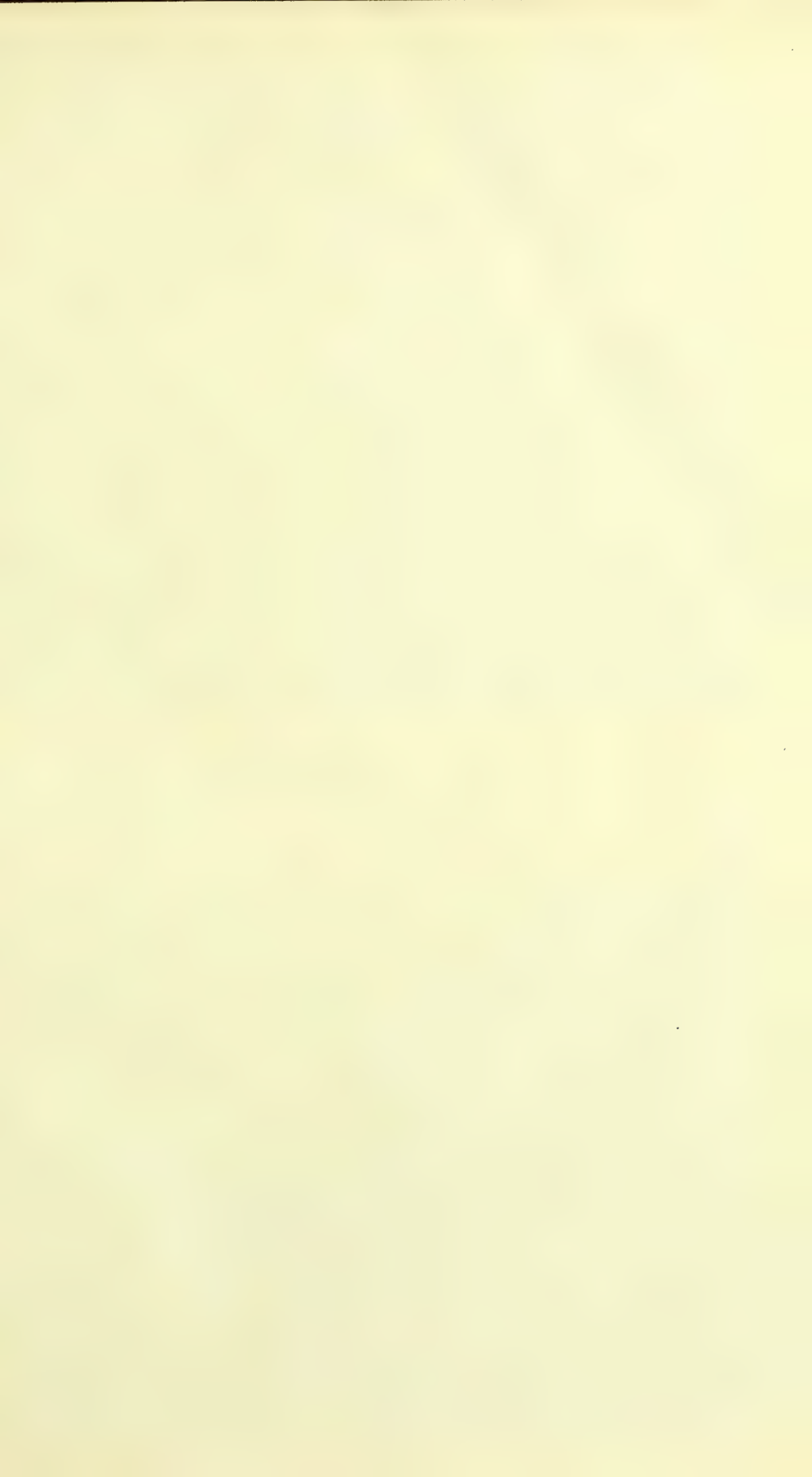
The work done by the Commission in Nyasaland, from the 12th January, 1912, until the 25th March, 1914, is given in the Table of Contents of this volume. Several of the papers written by the Commission have already been published in the Reports of the Sleeping Sickness Commission, No. XV. The titles of these are given in the Table of Contents and a short summary of each in the text of the present volume.

The Director was summoned to England in 1913 to give evidence before the Sleeping Sickness Committee, which had been constituted by Mr. Harcourt, the Secretary of State for the Colonies. The Committee was to meet in July under the chairmanship of the Rt. Hon. Mr. Alfred Lyttelton, but on account of his untimely death some delay took place, the first meeting of the Committee sitting on the 10th October, under the chairmanship of Lord Desart. This necessitated the absence of the Director and Lady Bruce from Kasu Camp from the 10th June until the 13th December, 1913.

Major Hamerton, who had been suffering from a good deal of malaria and was far from well, left on leave of absence on the 11th November, 1913, nine days after Captain Watson's arrival, so that when the Director returned to Kasu in December he found the work of the Commission practically at a standstill.

The Commission finally left Kasu on the 26th March, 1914, and arrived in England on the 17th May, 1914, the period of three years which was laid down as the probable duration of the enquiry having come to an end on the 31st March, the end of the third financial year.

The Commission takes this opportunity of expressing its sincere thanks to the Government officials and other gentlemen who assisted it in various ways. In the first place, the thanks of the Commission are especially due to H.E. Sir George Smith, K.C.M.G., the Governor





ANTELOPE ENCLOSURE. MARKET. DESTRUCTOR.
 SMALL ANIMAL HOUSE. STABLE. GOAT HOUSE.

OF KASU CAMP.



Fig 3.

of Nyasaland. Nothing could exceed the kindness and hospitality which His Excellency and Lady Smith extended to the Commission.

The thanks of the Commission are also especially due to Major F. B. Pearce, C.M.G., the then Deputy Governor, now British Resident, Zanzibar; Mr. W. Kirby Green, the Resident Magistrate of the Dowa District, who was of the greatest assistance in helping Major Hamerton to choose a site for the camp, which proved to be admirably adapted for the work; Dr. H. Hearsey, the Principal Medical Officer, and Drs. M. Sanderson, J. O. Shircore, J. E. S. Old, J. G. Morgan, and P. C. Conran, of the Nyasaland Medical Staff; Mr. T. I. Binnie, Director, and Mr. E. F. Bryan, Public Works Department; Mr. V. T. Keyte, Chief Transport Officer; Mr. G. Garden, Veterinary Department; Mr. S. Hewitt-Fletcher, British Consul at Chinde, and Mr. G. H. V. Mercier, Vice-Consul; the Revd. W. H. Murray, Dutch Reformed Church Mission, Mvera, Dowa District, Dr. W. A. Murray, and the other members of the Mission; and Mr. A. Johnstone, General Manager, Shiré Highlands Railway.

The Commission also received much help, and often of a difficult nature, from Sir Arnold Theiler, K.C.M.G., Director of Veterinary Research, Union of South Africa, Pretoria, and Mr. W. Robertson of the same department; also from Dr. F. K. Kleine, Director of the German Sleeping Sickness Commission, Ujidi, Lake Tanganyika, German East Africa, and his colleague, Dr. M. Taute.

In conclusion, the Commission desires to express its appreciation of the assistance rendered to it by Staff-Sergeant A. Gibbons, R.A.M.C., and Mr. James Wilson, in the work at Kasu. Staff-Sergeant Gibbons was a member of the Royal Society's Commissions to Uganda in 1903 and again in 1908. Mr. Wilson was also a member of the latter Commission. As on former occasions, Staff-Sergeant Gibbons was unceasing in activity. He is a non-commissioned officer of great versatility, and can turn his hand to anything from microscopical drawing to making roads or building houses. Mr. Wilson was also most willing and energetic. He was paymaster and had charge of the correspondence and accounts. In addition, he was bookkeeper and entered all observations made by the other workers into the ledger. In all these ways he was most persevering and trustworthy, and the Commission owes both these members a debt of gratitude for their unwearying and loyal services.

DEFINITION OF TERMS USED IN THIS REPORT.

Trypanosoma brucei.—The parasite found in the wild game and tsetse flies of Nyasaland and many other parts of Africa. It gives rise in man to the "Nyasaland sleeping sickness," and in other animals to the disease known as Nagana. In this Report it will be designated *T. brucei vel rhodesiense*.

Trypanosoma gambiense.—This parasite has been found in the blood of cattle and of antelope on the shores of Victoria Nyanza. It gives rise to the sleeping sickness of West Africa and Uganda the "Uganda sleeping sickness."

Sleeping Sickness.—There are two varieties of this disease in Africa. They will be distinguished in this Report by calling the one the "Uganda sleeping sickness," or "*T. gambiense* disease,"

and the other the "Nyasaland sleeping sickness," or "*T. brucei* vel *rhodesiense* disease." It would probably lead to less ambiguity if all infectious diseases were called by the name of the parasite causing them. For example, malarial fever is an ambiguous term, whereas the names "*Plasmodium malariae* fever," and "*Plasmodium vivax* fever" show definitely what kind of malarial fever is being dealt with.

Tsetse fly; the fly.—The principal carrier of the trypanosome diseases in Africa. The "Uganda sleeping sickness," or "*T. gambiense* disease," is carried by *Glossina palpalis*; the "Nyasaland sleeping sickness," or "*T. brucei* vel *rhodesiense* disease," by *Glossina morsitans*. It is possible in a laboratory experiment to infect animals with *Trypanosoma gambiense* by means of *Glossina morsitans* and with *Trypanosoma brucei* vel *rhodesiense* by means of *Glossina palpalis*, but this has never been proved to occur in Nature.

Fly-country.—Any belt or tract of country where tsetse flies abound.

ANATOMY OF TSETSE FLIES.

Proboscis.—The piercing apparatus of the tsetse fly.

Labial cavity.—This is the tube in the proboscis made by the bringing together of the two parts, the labium and the labrum. The labial cavity is the tube through which the blood passes to the stomach when the fly is feeding.

Hypopharynx.—The terminal duct of the salivary glands. It runs in a groove in the floor of the labial cavity into which it opens near the tip of the proboscis. It conveys the secretion of the salivary glands.

Salivary glands.—Large glands which lie principally in the abdomen of the tsetse fly. They secrete a fluid which, when injected into an animal, causes irritation and a flow of blood to the bitten point. This enables the fly to obtain a plentiful supply of food.

Alimentary system.—The digestive tube of the fly, comprising the labial cavity, the proventriculus, the sucking stomach, the fore, mid, and hind gut, and the rectum.

Proclaimed Area.—The area in Nyasaland where most of the cases of "Nyasaland sleeping sickness," or "*T. brucei* vel *rhodesiense* disease," have occurred. It is also known as the Sleeping Sickness Area (see Map 1, p. 1).

Infected and Infective flies.—An infected fly is one in which development of the trypanosomes has taken place, but unless the development has reached a certain stage the fly is not infective. An infective fly is one which is capable of conveying infection. An infective fly must be infected, an infected fly need not be infective.

Blood forms of trypanosomes.—The trypanosomes in the blood of an infected animal are very different in size and shape from the same trypanosomes undergoing development in the tsetse fly. When the development is complete, however, the trypanosomes revert to the blood form, and it is only when the fly contains these blood forms that it is infective.

SUMMARY OF THE WORK DONE BY THE COMMISSION AND SOME PRACTICAL RECOMMENDATIONS WHICH ARISE OUT OF IT.

As the terms of reference given to the Commission were to enquire into the relation of the African fauna to the maintenance and spread of trypanosome diseases in man and domestic animals, one of the first investigations carried out by the Commission was the examination of the wild animals living in the Proclaimed Area. The result of this examination showed that about one-third of the antelope living in this area were infected with various trypanosomes. The same trypanosomes were also found later in the wild animals living in the fly-belt of the Upper Shire valley to the south of the Proclaimed Area (see Map 3, facing p. 216). An examination of the smaller mammals, including monkeys, in the Sleeping Sickness Area, however, gave negative results. Another important point in this connection is that the wild game living outside the fly-country were found to be harmless, not a single pathogenic trypanosome having been observed in their blood.

The tsetse flies (*Glossina morsitans*) were next examined, and it was found that they were heavily infected with the same species of trypanosomes as those found in the blood of the wild game. The fly in the Upper Shire valley was also found infected in the same way.

An examination of the other important species of tsetse fly found in Nyasaland, *Glossina brevipalpis*, also showed that they were infected with the same trypanosomes, and probably are just as capable as *Glossina morsitans* of spreading these diseases.

The trypanosomes with which the wild game and the fly were infected were found to belong to four species—*Trypanosoma brucei* vel *rhodesiense*, *Trypanosoma pecorum*, *Trypanosoma simia*, and *Trypanosoma capræ*.

One important conclusion which the Commission has tried to establish is that *Trypanosoma brucei*, the cause of Nagana in Zululand, and other parts of Africa, is identical with *Trypanosoma rhodesiense*, the trypanosome causing disease in man in Nyasaland and Rhodesia. In this Report, this species is, therefore, designated *Trypanosoma brucei* vel *rhodesiense*. *Trypanosoma brucei* vel *rhodesiense* was studied in man, in the wild game, in the wild *Glossina morsitans*, and from several other sources. In all, 28 strains of this trypanosome were worked with, and the conclusion was arrived at that all of them belong to one and the same species.

Trypanosoma pecorum, the commonest of the pathogenic trypanosomes in Nyasaland, affects cattle and other domestic animals, and is probably the most important and dangerous species of all those which affect the stockowner.

A new species of trypanosome—*Trypanosoma simia*—is also described in the Report, which is chiefly remarkable for the rapidity with which it kills the domestic pig.

Three strains of *Trypanosoma gambiense* sent by Dr. Kleine from Lake Tanganyika were investigated. The chief point of difference between this species of trypanosome and *Trypanosoma brucei* vel

rhodesiense is its want of virulence towards experimental animals. There is also a scarcity or absence of the blunt-headed, posterior-nucleated forms, which are so common a feature in the blood of experimental animals infected with *Trypanosoma brucei* vel *rhodesiense*. And further, the disease in man caused by the last-named trypanosome appears to be much more rapid and acute than that caused by *Trypanosoma gambiense*.

Practical recommendations.—On account of the marked infectivity of the wild game in the fly-country—and this fact stands out most prominently and without any shadow of doubt—the Commission recommended that efforts should be made to diminish, as far as possible, the number of wild animals in fly-areas. Every wild animal destroyed or driven out is one less source or centre of infection. It was also considered likely that as the tsetse fly found its chief source of food supply in the wild game, any diminution of these animals would lead to a corresponding diminution in the number of the flies.

As to the means of bringing about this diminution of wild game in fly-areas, the Commission is aware of the great difficulties lying in the way. It is not practicable to arm the natives to any extent with arms of precision. Now that it is known that man is liable to be attacked by this form of trypanosome disease, it is not likely that many white hunters will enter this district on purely sporting trips. The Proclaimed Area used to be a favourite hunting locality, but while the Commission was at Kasu no shooting parties were known to enter it. No doubt if the Government offered a fixed remunerative price for every skin brought in, professional hunters would be found to go into the fly-areas and bring about a marked lessening in the numbers of the game. The Commission recommended that all restrictions should be removed regarding the pursuit and killing of wild game in fly-areas. The natives should be allowed to pursue any of the ordinary wild animals without let or hindrance. They might use their own methods, such as game-pits or traps, and they should be permitted to use a certain number of useful guns. The wild game in fly-country may be said to be all infected, and the fewer of them about the better for man and his domestic animals.

Segregation of infected natives.—Every infected native in the fly-country is a source of danger to his neighbours. If the trypanosome causing disease in man in Nyasaland is in truth the common *Trypanosoma brucei* of the wild game, which under certain conditions can infect human beings, then *Trypanosoma brucei* taken by a fly from an infected man would be more likely to infect man than a *Trypanosoma brucei* taken from a wild animal. It would have become acclimatised to the blood of man just as *Trypanosoma gambiense* has become. Therefore it would be well to move infected natives out of the fly-country into fly-free parts. This could be done without isolating him in a segregation camp. These camps are very unpopular, as no one who goes in ever comes out alive. The sick native might be sent to some neighbouring village out of the fly-country and attended to by his own relatives. This, however, is not an important matter. The sick are not usually discovered until they are seriously ill, when removal would be impossible, and infected natives are few and far between.

Clearing of forest around villages.—This is thought to be useful from many points of view. It helps to keep the fly away from the village proper, so that if there is an infected native there, there will be less chance of his being bitten and the tsetse flies becoming infected from man. It lets in sunlight and air and keeps the wild animals at arm's length. For purposes of administration, it would be well also to gather natives together in fairly large villages, rather than allow them to live in single huts scattered through the bush.

Prophylaxis and treatment by means of drugs.—This, with our present knowledge, the Commission considers to be of little or no practical use.

Destruction of the tsetse fly.—In the opinion of the Commission the problem cannot be attacked with any chance of success from the side of the fly alone. The fly-country in Nyasaland comprises some 5,000 square miles, and much of it is desert. The tsetse flies are numerous and deposit their larvæ all over the country. It seems to be quite impracticable to lessen the number of tsetse flies to any material extent by killing either them or their pupæ. When the country becomes opened up, cleared and settled, the big game will disappear and the tsetse with them. Until then, it would appear to be the best policy to keep down the number of wild game in fly-areas as much as possible and to clear as much space round villages as is practicable.

A.—EXAMINATION OF THE NYASALAND FAUNA.

HISTORICAL.

In 1894, in Zululand, it was discovered for the first time that the "tsetse-fly disease," or Nagana, was caused by a blood parasite belonging to the protozoa, a flagellate called by the generic name *Trypanosoma*. The particular species of trypanosome encountered in Zululand in 1894 was one which was giving rise at that time to a serious epidemic among the native cattle. This species, which was afterwards called *Trypanosoma brucei* by Plimmer and Bradford, is particularly deadly to horses, mules, donkeys, and dogs, but not so much so to oxen, sheep, and goats. In Zululand at that time it was thought that a single species of trypanosome was being dealt with, but now with further knowledge it is clear that there was also present another species—*Trypanosoma pecorum*—a much smaller parasite and one particularly fatal to cattle, sheep, and goats.

It was proved at that time, and for the first time, that these parasites were carried from sick to healthy animals by the local tsetse fly (*Glossina morsitans*).

As no healthy horse or dog could be exposed even for a few hours in the Zululand fly-country, and as there were no horses or other domestic animals in this fly-country, it was evident that the fly must procure the virus somewhere, and this led to the examination of the blood of the wild animals living in this district. In the blood of these animals—buffalo, koodoo, etc.—the same trypanosome was found, and thus was established the fact that the wild game act as a reservoir of the virus of the trypanosome diseases.

This work was done in Zululand between the years 1894 and 1897, and the following list represents the result of the examination of some of the wild animals found in the neighbouring fly-belt:—

Animal.	Total No. examined.	No. found infected.
Buffalo	8	1
Koodoo	8	4
Wildebeeste	13	3
Waterbuck	2	0
Impala	2	0
Zebra	2	0
Rushbuck	1	1
Steinbuck	2	1
Warthog	1	0
Wild pig	1	0
Hyena	1	1
Reedbuck	1	1
Leopard	2	0
Duiker	1	0
Roi Rei buck	2	0
Mongoose	1	0
Jackal	1	0
Total	49	12

Percentage of infected animals—24 per cent.

Since 1897, some further observations have been made regarding the presence of these parasites in the blood of wild game on the West Coast of Africa, in Uganda, and notably in North Eastern Rhodesia by Kinghorn and Yorke.

I.—THE TRYPANOSOMES FOUND IN THE BLOOD OF WILD ANIMALS LIVING IN THE SLEEPING SICKNESS AREA, NYASALAND.

This paper has already been published in the "Proceedings" of the Royal Society, B, volume 86, 1913, page 269, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., page 16.

It was found that 31·7 per cent. of the wild game in the fly-country below Kasu Hill, harbour pathogenic trypanosomes. The species found are *Trypanosoma brucei* vel *rhodesiense* 7·8 per cent., *Trypanosoma pecorum* 14·4, *Trypanosoma simia* 1·7, *Trypanosoma capre* 11·1, and *Trypanosoma ingens* 1·7.

Although only 31·7 per cent. of the wild animals examined showed trypanosomes in their blood, it is probable that a much larger percentage is infected. It may, in fact, be said that in all probability all the wild game in the fly-country are infected. When it is considered how scarce the trypanosomes may be in the blood of these animals, it is not to be wondered at if some are passed as clean when they are really infected. It would seem impossible that any of the large wild animals living in herds as they do, and constantly preyed on by innumerable tsetse flies, should escape infection at one time or another.

From this it would seem to be self-evident that these wild animals should not be allowed to live in fly-country, where they constitute a standing danger to the native inhabitants and the domestic animals. It would be as reasonable to allow mad dogs to live and be protected by law in our English towns and villages. Not only should all game laws restricting their destruction in fly-country be removed, but active measures should, if feasible, be taken for their early and complete blotting out. It must be strictly borne in mind that this only refers to wild animals living in fly-areas. No pathogenic trypanosomes have, up to the present, been found by the Commission in the blood of animals living in fly-free areas.

II.—TRYPANOSOMES FOUND IN THE BLOOD OF WILD GAME LIVING IN THE UPPER SHIRÉ RIVER FLY-BELT.*

In June, 1913, one of the members of the Commission went to the Liwonde District to identify and isolate the various species of trypanosomes infecting the fly and wild game in the fly-belt which extends along the Upper Shiré River valley from Lake Pamalombe to the Murchison cataracts. This fly-area is, roughly speaking, 100 miles south of Kasu and the Proclaimed Area. It is separated from the extensive fly-area of the plains on the west shore of Lake Nyasa, of which the Proclaimed Area forms a part, by a range of hills and high plateaux where the fly is absent, although there is nothing to prevent trypanosome-infected wild animals wandering from one fly-belt into the other.

The valley of the Upper Shiré is thickly populated, and the fly-area is crossed by two of the most frequented roads in Nyasaland: the grand trunk road running from Zomba to the north and the main road from Liwonde to Fort Johnston. Although thickly populated, human trypanosome disease, though probably existing, has not yet been discovered in this district.† The natives, however, can keep no cattle, and their goats and dogs are constantly destroyed by trypanosome diseases, so that they have to continually import these animals from the highlands.

Game is very abundant in this district, particularly in the dry season, when herds of eland, koodoo, waterbuck, and impala concentrate in the vicinity of the river. In the wet season, elephant and buffalo wandering about the country, frequently remain for many weeks in the impenetrable thickets and swampy "dambos" along the river banks.

A characteristic feature of the flora of this district is the extensive forests of "sanya" trees, open forests of medium-sized trees, devoid of undergrowth, but carpeted with short wiry grass. Large herds of impala are always to be found in these forests, and tsetse flies are everywhere, being particularly numerous along the dusty tracks made by the antelope and around the pools where they drink.

Nandumba's village (latitude 14° 40' S., longitude 30° 10' E.), on the banks of the Shiré River, was selected as the locality for the camp in which to carry out experiments of feeding flies on healthy dogs and goats, monkeys being unobtainable. The experiments were carried out between the dates of 19th June and 25th July, 1913.

* Reprinted, with amendments, from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 40.

† Since this was written cases of trypanosome disease in man have been found.

*Examination of the Blood of Wild Animals in the Upper Shire
River Fly-Belt.*

Whenever wild animals were killed their blood was examined for trypanosomes, which were identified by the microscope in stained films of the blood. The following table gives the results:—

Table I.

Animal.	Species of trypanosomes found.				
	<i>T. brucei</i> vel <i>rhodesiense</i> .	<i>T. pecorum</i> .	<i>T. simia</i> .	<i>T. capræ</i> .	<i>T. ingens</i> .
Zebra	—	—	—	—	—
Impala	—	—	—	—	—
Impala	—	—	—	—	—
Impala	—	—	—	+	—
Impala	—	—	—	—	—
Impala	—	+	—	—	—
Impala	—	+	—	—	—
Impala	—	—	—	—	—
Koodoo	—	—	—	—	—
Koodoo	—	—	—	+	—
Waterbuck	—	—	—	—	—
Waterbuck	—	+	—	—	—
Waterbuck	—	+	—	—	—
Waterbuck	+	—	—	—	+
Waterbuck	—	—	—	—	—
Waterbuck	—	—	—	+	—

This table, even in so small a series of animals examined, indicates that *Trypanosoma pecorum* occurs frequently in the wild game, such as the impala and waterbuck. *Trypanosoma brucei* vel *rhodesiense* was found in only one animal out of the 16, *Trypanosoma simia* in none, *Trypanosoma capræ* in three, and *Trypanosoma ingens* in one.

III.—EXAMINATION OF SMALL MAMMALS LIVING IN THE SLEEPING SICKNESS AREA, NYASALAND.

A few small mammals captured in the Sleeping Sickness Area were examined for trypanosomes, but in not a single case were any of the mammalian pathogenic trypanosomes found in their blood. In the wild field rat *Trypanosoma lewisi* was present, and in the jumping shrew a new trypanosome, to which the name *Trypanosoma petrodromo* has been given, was fairly common.

Table II.

Species of animal examined.	Number examined.	Trypanosomes found in.	Remarks.
Lemur	4	0	
Honey badger (<i>Mellivora ratel</i>)	2	0	
Ant-bear	1	0	
Mongoose (<i>Mungos mclanrus</i> zomba, Wrought, and <i>Crossarchus fasciatus</i> , Desm.) ...	7	0	
Squirrel (<i>Paracerus cepapi</i> , Sm.)	13	0	
Hare	2	0	
Shrew (<i>Petrodromus venustus</i> , Thos.)	77	5	<i>T. petrodromo.</i>
Mouse	1	0	
Wild cat	6	0	
Wild rat	10	3	<i>T. lewisi.</i>
Edible rat (<i>Cricetomys gambianus viator</i> , Thos.)	2	0	
Baboon	12	0	
Black-faced monkey (<i>Cercopithecus pygerythrus whitei</i> , Thos.) ...	400	0	

Conclusion.

The small mammals living in the Sleeping Sickness Area are not infected by pathogenic trypanosomes, and their rôle in the spread of trypanosome disease to man and the domestic animals is probably infinitesimal and may be ignored.

IV.—EXAMINATION OF WILD GAME IN FLY-FREE AREAS.

The blood of the wild animals shot in fly-free areas was examined microscopically for trypanosomes. In no case was a single pathogenic trypanosome found. If, in addition to the microscopical examination, a quantity of blood had been injected into susceptible animals, it is possible that in some cases trypanosomes might have been proved to be present. It will be remembered that, in Uganda, antelope still remained infective long after the trypanosomes could no longer be seen in the blood by the microscope.* But it was impossible in Nyasaland to use this method of finding trypanosomes in the blood of the wild game living in fly-free areas, as the great majority of the animals were shot on the Bua River and round Fort Manning and Fort Jameson, 100 miles or so to the west of the Proclaimed Area.

* "Further Investigations on the Rôle of Antelope as a Reservoir of *T. gambiense*." Dr. H. L. Duke. "Reports of the Sleeping Sickness Commission, No. XIII., p. 58.

The following is a list of the animals shot in fly-free areas and found to be free from trypanosomes:—

Table III.

Species of animal.	No. examined.
Elephant	1
Hippopotamus	1
Koodoo	3
Sable	7
Zebra	6
Eland	7
Roan	13
Waterbuck	7
Hartebeeste	5
Puku	4
Reedbuck	17
Oribi	2
Duiker	5
Warthog	6
Antelope (unclassified)...	29
Total	113

V.—EXAMINATION OF DOMESTIC ANIMALS LIVING IN THE SLEEPING SICKNESS AREA, NYASALAND.

Goats.—The blood of 140 goats from different villages in the fly-country was examined for trypanosomes. Six were shown to be infected—4 per cent. In five cases the trypanosome found was *Trypanosoma pecorum*, and in the remaining one *Trypanosoma capræ*.

Dogs.—The blood of 22 dogs was also examined microscopically; of these eight were found to be infected—36 per cent.—six with *Trypanosoma pecorum* and two with *Trypanosoma brucei* vel *rhodesiense*, the trypanosome causing disease in man in Nyasaland.

Conclusion.

It must be admitted that the domestic animals living in the Sleeping Sickness Area, Nyasaland, may act to some extent as reservoirs of the disease, but their number in fly-areas is so small that they probably constitute little or no danger.

B.—EXAMINATION OF TSETSE FLIES IN NYASALAND.

INTRODUCTORY.

The tsetse flies reported to be found in Nyasaland are *Glossina morsitans*, *pallidipes*, and *brevipalpis*. The most important and most widely distributed of these is *Glossina morsitans*.

Glossina palpalis, the carrier of Congo sleeping sickness, or *Trypanosoma gambiense* disease, has not been found.

The distribution of the tsetse flies in Nyasaland is given on Map 3, p. 216. *Glossina pallidipes* may be ignored, as it is very rare and has the same appearance and habits as *Glossina morsitans*.

Tsetse flies are dull-coloured, ordinary-looking flies, about half an inch in length. They have a powerful proboscis which, when not in use, projects horizontally in front. The wings are long and close over each other like the blades of a pair of scissors, when the fly is at rest. The upper side of the abdomen is marked by five more or less distinct transverse abdominal bands. Their method of reproduction is different from that of the ordinary fly: tsetse flies do not lay a number of eggs like the majority of the Diptera, but they lay a single larva which immediately after birth creeps into the nearest cranny among loose sand, earth, or dead leaves, and there turns into a black and hard puparium. Five or six weeks afterwards the adult fly emerges.

Glossina morsitans lives in the dry thorny scrub which covers large areas of tropical Africa, and is the most widely distributed of the tsetse flies. It is not as a rule found along the banks of rivers or lakes. It is much more numerous towards the end of the rainy season and diminishes in numbers towards the end of the dry season when all the grass is burnt off. It occurs in some places in great numbers and attacks with great voracity, resembling a swarm of angry bees. It is more attracted by a moving than a stationary object, and it is when riding a motor-cycle through fly-country that the greatest number attacking at the same time has been seen.

Glossina brevipalpis is much larger than *Glossina morsitans*. It is found in Nyasaland along the banks of the numerous streams which run into the Lake. It is infected with pathogenic trypanosomes in the same way as *Glossina morsitans*, and is equally capable of acting as carrier.

VI.—INFECTIVITY OF *GLOSSINA MORSITANS* IN THE SLEEPING SICKNESS AREA, NYASALAND.

This paper has already been published in the "Proceedings" of the Royal Society, B, volume 86, 1913, page 422, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., page 73.

The result of the examination of 10,081 tsetse flies (*Glossina morsitans*) caught in the fly-country near Kasu was, that the proportion of infective flies to non-infective was found to be 13·5 per 1,000, or 1 in 74.

They were infected with four species of pathogenic trypanosomes—*Trypanosoma brucei* vel *rhodesiense*, *Trypanosoma pectorum*, *Trypanosoma simia*, and *Trypanosoma capræ*.

In regard to *Trypanosoma brucei* vel *rhodesiense*, the trypanosome causing disease in man in Nyasaland, it was found that 2 per 1,000 of the flies were infective with it.

It was also established that the flies are infective all the year round.

VII.—PERCENTAGE OF INFECTED *GLOSSINA MORSITANS* IN THE SLEEPING SICKNESS AREA, NYASALAND, DURING 1912 AND 1913.*

The object of this paper is to attempt to set up a rough standard of the proportion of infected to non-infected tsetse flies in an ordinary fly-area where wild game abounds. It was thought that a standard of this kind might prove useful in the future.

The flies were collected in the low-country lying near the Commission's camp at Kasu, in what is known as the Proclaimed or Sleeping Sickness Area of Nyasaland. This part of the country swarms with *Glossina morsitans* and wild game, the latter highly infected and well protected.

The flies when brought to the laboratory were not fed on animals, but merely dissected and examined microscopically. By this means it is possible to tell the proportion of infected flies.

In 1912, a total of 1,975 flies were dissected between the months of January and November. Of these 129 were found to be infected with trypanosomes—6·53 per cent. Males, 86 per cent.; females, 14 per cent.

In 1913, 1,060 flies were dissected, of which 91 were infected—8·58 per cent.

* Reprinted, with additions, from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 43.

The following tables give the details:—

Table IV.—1912.

No. of fly.	Pro-bos-cis.	Pro-ventri-culus.	Crop.	Intes-tine.	Salivary glands.	Part of fly injected.	Animal in-jected.	Result.
1	+	+	+	+				
2	+	+		+		Intestine	Dog	
3			+	+		"	"	
4				+		"	Goat	
5				+		"	Dog	
6				+		"	Goat	
7		+		+				
8	+	+		+		"	Dog	
9	+	+				Proboscis	Goat	
10	+	+		+		"	"	
11				+				
12	—			+	—			
13	—			+	—	Intestine	Dog	—
14	+			+	—			
15	—	—		+				
16				+				
17	+			+				
18				+				
19	+			+				
20				+				
21				+				
22	+			+				
23				+				
24	+	+		+				
25	+	+		+				
26	—			+				
27	+	+	+	+				
28	+			+				
29				+		Intestine & Sal. glands	"	
30				+		Intestine	"	—
31				+				
32	+			+		Intestine Proboscis	"	—
33	+	+		+				
34				+		Intestine	Goat	—
35				+		"	Dog	—
36				+		"	Goat	—
37		+		+		Intestine & Sal. glands	"	
38				+		Intestine	"	—
39	+					Proboscis	"	—
40	+			+		Intestine & Proboscis	Dog	—
41				+		Intestine	"	—
42	+					Proboscis	Goat	—
43				+		Intestine	"	—
44	+					Proboscis	"	—
45				+		Intestine	"	—
46				+		"	Dog	—
47	+					Proboscis	Goat	—
48				+		Intestine	Dog	—
49	+	+		+		Intestine Proboscis	"	—

Table IV.—1912 (*continued*).

No. of fly.	Pro-bos-cis.	Pro-ventri-culus.	Crop.	Intes-tine.	Salivary glands.	Part of fly injected.	Animal in-jec'ted.	Result.
50	+			+		Intestine	Dog	—
51	—	—		+	—	Proboscis	Goat	—
52	+			+		Intestine	Dog	—
53	+			+		"	"	—
54	—	—	—	+		Proboscis	"	—
55	+			—		Intestine	"	—
56	—			+		Proboscis	Goat	—
57	—			+		Intestine	"	—
58	—			+		"	"	—
59	—			+		"	"	—
60	—			+		"	"	—
61	+			—		"	"	—
62	+			+		"	"	—
63	—	+	+	+				—
64	+			—				—
65	—			+				—
66	+			+				—
67	+			—				—
68	+			—				—
69	+			+				—
70	—	—		+				—
71	+			+				—
72	—	+	+	+		"	Monkey	—
73	+	+	+	+				—
74	—	+	+	+				—
75	—		+	+				—
76	+	—		+				—
77	+			+		Proboscis	"	—
78	+			+				—
79	+			+				—
80	+			—				—
81	—		—	+				—
82	+			+				—
83	+			+				—
84	—	+	+	+				—
85	—	—		+				—
86	—		+	+				—
87	+			—				—
88	+			—				—
89	—			+				—
90	—			+				—
91	—			+				—
92	+			—				—
93	—			+				—
94	+	+	+	+				—
95	—			+				—
96	—	+		+				—
97	+			+				—
98	+			+				—
99	—			+				—
100	—			+				—
101	—			+				—
102	+			+				—
103	—	+	+	+				—
104	+	+	+	+				—

Table IV.—1912 (continued).

No. of fly.	Pro-bos-cis.	Pro-ventri-culus.	Crop.	Intes-tine.	Salivary glands.	Part of fly injected.	Animal in-jected.	Result.
105	—			++				
106	+			++				
107	++			++	—	Intestine	Dog	—
108	++			++	—			
109	+	++		++		„ & Proboscis	Goat	+ <i>T. simiae.</i>
110	++	—		—		„	„	—
111	+	++	++	++		„ & Intestine	Dog	—
112	—			++	—	„	„	—
113	—			++	—	„	„	—
114	—	++		++				
115	—			+				
116	—			+	—			
117	—			++				
118	+	++	++	++	—	Intestine & Sal. glands	„	—
119	—			++		Intestine	„	—
120	+			—				
121	+		—	++		„	„	—
122	—	++	—	++	—			
123	+		+	++	—	„	„ & Goat	—
124	+		++	++	—			
125	—			+		„	Dog	—
126	—		—	++	—	„	Goat	—
127	+		+	+	—			
128	—	+	—	++	++	Sal. glands	Rat	+ <i>T. brucei</i> <i>vel rhodesiense.</i>
129	—	+	—	++	++	„	„	+ <i>T. brucei</i> <i>vel rhodesiense.</i>

It will be seen from the above table that 60 attempts to determine the infectivity of the flies were made by injecting emulsions of the infected organs into healthy animals. In only three cases did the animals become infected: once with *Trypanosoma simiae* and twice with *Trypanosoma brucei vel rhodesiense*. The usual experiment was to inject the contents of the intestine into dogs or goats. This is now known to be useless, as the developmental forms in the intestine are not infective. Doubtless more positive results could be got with more knowledge of the laws which govern infectivity. Only in two cases were the salivary glands found to be invaded. This infection, of course, could only be *Trypanosoma brucei vel rhodesiense*, and this was confirmed by injecting the glands into rats.

In 1912, no attempt was made to diagnose directly the species of trypanosomes with which the flies were infected, but in 1913 this

was done, as by that time a good deal of experience had been gained. For example, invasion of the salivary glands could only be *Trypanosoma brucei* vel *rhodesiense*; invasion of the intestine, labial cavity, and hypopharynx meant *Trypanosoma pecorum*, or *Trypanosoma simia*, and size would distinguish between the two. Lastly, if only the labial cavity and hypopharynx were seen to contain flagellates, then *Trypanosoma capræ* was indicated, and here also the size and character of the trypanosomes in the hypopharynx would assist in the diagnosis.

Table V.—1913.

No. of fly.	Proboscis.		Intestine.	Salivary glands.	Species of trypanosome.
	Labial cavity.	Hypopharynx.			
1	+		+		<i>T. pecorum.</i>
2	+		—		<i>T. capræ.</i>
3	+		—		"
4	+		—		"
5	+		+		<i>T. simia.</i>
6	+		—		<i>T. capræ.</i>
7	—	+	+	—	
8	—	—	+	—	
9	+		+		<i>T. simia.</i>
10	+		—		<i>T. capræ.</i>
11	+		—		"
12	—		++	—	
13	—		+	—	
14	—		+	—	
15	+		++		<i>T. simia.</i>
16	+		+		"
17	+		+		<i>T. capræ.</i>
18	+	+	+	—	<i>T. pecorum.</i>
19	+		+		
20	+		+	—	
21	—	—	++	+	<i>T. brucei.</i>
22	—		+	—	
23	+		—		<i>T. capræ.</i>
24	+		—		"
25	—		+		
26	—		+	—	
27	+	+	+	—	
28	—		+	—	
29	—		+	—	
30	—		+	—	
31	+		+	—	<i>T. pecorum.</i>
32	+		+		<i>T. simia.</i>
33	—		+		
34	—		+		
35	—		+	—	
36	+		+		<i>T. simia.</i>
37	—		+	—	
38	+		+	—	<i>T. pecorum.</i>
39	+		+	—	<i>T. simia.</i>
40	+		+	—	"
41	+		—	—	<i>T. capræ.</i>
42	—		+	—	

Table V.—1913 (*continued*).

No. of fly.	Proboscis.		Intestine.	Salivary glands.	Species of trypanosome.
	Labial cavity.	Hypo-pharynx.			
43	—		+	—	
44	+		—		<i>T. capre.</i>
45	—		+		
46	—		+	—	
47	+		—		"
48	—		+	—	
49	—		+	—	
50	—		+	—	
51	—		+	—	
52	+		+		
53	+		—		
54	+		—		
55	—		+		
56	+	+	+		
57	+	—	—		
58	+	—	—		
59	—		+		
60	+	+	+		<i>T. simia.</i>
61	+	—	+		"
62	—		+		
63	+	—	—		<i>T. capre.</i>
64	+	+	+		<i>T. pecorum.</i>
65	—		+		
66	+	+	—		<i>T. capre.</i>
67	—	—	+		
68	+	—	—		
69	+	—	—		
70	—		+	—	
71	+	—	—		
72	+	—	—		
73	—		+		
74	+	—	—		
75	+	—	—		
76	+	—	+	—	
77	+	—	—		
78	+	—	—		
79	—		+	—	
80	+	—	+		<i>T. simia.</i>
81	—		+		
82	+	—	—		
83	+	—	+		<i>T. pecorum.</i>
84	+	—	+		<i>T. simia.</i>
85	—		+	—	
86	+	+	—		
87	+	—	—		
88	+	—	—		
89	+	—	+	—	
90	—	—	+	—	
91	—	—	+	—	

In 1913, no injections of the contents of organs were made into healthy animals. The direct diagnosis of the species of trypanosome by examination of the fly took the place of inoculation.

From the above table it will be seen that in 1,060 flies, *Trypanosoma brucei* vel *rhodesiense* was found once, *Trypanosoma pecorum* six times, *Trypanosoma simiae* 12 times, and *Trypanosoma capræ* 14 times. It must, however, be confessed that the margin of error in this calculation is very great.

Conclusion.

In 1912, 6.53 per cent. of the *Glossina morsitans* found in the Proclaimed or Sleeping Sickness Area, Nyasaland, were infected with pathogenic trypanosomes; in 1913, 8.58 per cent.

VIII.—INFECTIVITY OF *GLOSSINA MORSITANS* IN THE UPPER SHIRÉ RIVER FLY-BELT.*

In a former paper† on the trypanosomes found in the wild game living in the Upper Shiré River fly-belt, it was explained that a member of the Commission spent some time in the Upper Shiré Valley, in order to discover if the wild game and wild *Glossina morsitans* found in that fly-belt were infected with the same species of trypanosomes as the fly in the Sleeping Sickness Area near Kasu camp.

The method employed in the feeding experiments was the same as described in a previous paper,‡ except that monkeys were unobtainable and the flies were only fed twice on each dog and goat.

All infected animals were subsequently taken to Kasu camp, the usual precautions being taken to prevent re-infection on the way, and the trypanosomes found in them were compared with the species and strains of trypanosomes obtained from human beings, various animals, and the tsetse flies in the Sleeping Sickness Area. Special attention and study were devoted to the comparison of the strain of the trypanosome causing disease in man in Nyasaland—*Trypanosoma brucei* vel *rhodesiense*—from the Upper Shiré Valley with strains obtained from human beings, various animals, and the tsetse flies in the Sleeping Sickness Area.

The following table gives in the first column the date the tsetse flies were first fed on the experimental animals, the second column the number of flies fed, and the signs plus and minus show the result of feeding the flies on the dog and goat.

* Reprinted, with additions, from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 38.

† "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 38, and p. 10, of this Report.

‡ "Infectivity of *Glossina morsitans* in Nyasaland." "Proceedings" of the Royal Society, B, vol. 86, 1913, p. 408, and Reports of the Sleeping Sickness Commission, No. XV., p. 73.

It will be seen that the fly in the Upper Shire District carries the same four species of trypanosomes as those found at Kasu in flies from the Proclaimed Sleeping Sickness Area: *Trypanosoma brucei vel rhodesiense*, *Trypanosoma pecorum*, *Trypanosoma simiæ*, and *Trypanosoma capræ*.

Table VI.—Infectivity of Wild *Glossina morsitans* in the Upper Shire River Fly-Area.

			Dog.				Goat.			
Date.	No. of flies fed.		<i>T. brucei vel rhodesiense.</i>	<i>T. pecorum.</i>	<i>T. simia.</i>	<i>T. capra.</i>	<i>T. brucei vel rhodesiense.</i>	<i>T. pecorum.</i>	<i>T. simia.</i>	<i>T. capra.</i>
1913.										
June 19	...	...	73	+	—	—	—	+	+	+
„ 26	...	...	150	+	+	—	—	+	—	+
July 2	...	...	100	+	—	—	—	+	—	+
„ 4	...	...	450	+	—	—	—	+	—	+
„ 8	...	...	650	+	+	—	—	—	—	+
„ 15	...	...	650	+	—	—	—	+	—	—
„ 22	...	...	500	—	+	—	—	+	—	—

Here, in a series of seven experiments, all the animals on which the flies were fed developed trypanosome disease. In six experiments the flies infected the dogs with *Trypanosoma brucei vel rhodesiense*; in the second and fifth there was a double infection with *Trypanosoma pecorum*; and in the seventh an infection with *Trypanosoma percorum* alone. None of the goats were infected with *Trypanosoma brucei vel rhodesiense*. Six goats were infected with *Trypanosoma pecorum*, one with *Trypanosoma simiæ*, and five with *Trypanosoma capræ*. It will be noticed that the smallest batch of flies used, a batch of 73, infected the dog with *Trypanosoma brucei vel rhodesiense*, and the goat with *Trypanosoma pecorum*, *Trypanosoma simiæ*, and *Trypanosoma capræ*.

Conclusions.

1. The trypanosomes found in the wild *Glossina morsitans* of the Upper Shire River fly-area are identical with those found 100 miles further north in the Proclaimed Area.

2. The trypanosome causing disease in man in Nyasaland—*Trypanosoma brucei vel rhodesiense*—is frequently met with, so that it is probable cases of this form of sleeping sickness will be found among the natives of this district. (As already stated, this was soon proved to be true by Dr. Sanderson discovering a case near Mpimbi.—Director.)

IX.—DESCRIPTION OF THE TSETSE FLY (*GLOSSINA MORSITANS*) WHILE FEEDING.

A guinea-pig was used as the feed, a patch being shaved on the abdomen. A wild fly, with its wings cut off, was placed on the shaved patch and his proceedings were watched through a microscope. Unfortunately, on this occasion, the fly elected to turn his back on the observer. He lost no time, but at once began vigorous up-and-down movements of the head and thorax, as though he found the skin tough to pierce. Whether these movements were performed for the purpose of boring or of sucking as well, it is impossible to say, owing to the observer's position. But, after half a minute, the guinea-pig made some violent struggles and the fly was displaced. He was replaced at once and proceeded to clean his proboscis with great care by passing his forelegs simultaneously down it from base to tip, freeing it from the protecting sheath of the palpi to do so. He also carefully cleaned the forelegs by rubbing one against the other, including the femora up to their junction with the thorax. This done, he appeared to plunge his proboscis suddenly and without difficulty through the skin, his back still towards the observer, and appeared to suck, without any visible movement, and after half a minute he was fully distended. The cleaning process was then repeated and he remained quiescent until removed.

Two further flies were fed in succession in a small glass box placed on the guinea-pig's abdomen. The first settled three-quarter front face to the observer and at once started the boring movements described above. The head and front part of the thorax were depressed between the widely-separated forelegs for a moment and then raised, when the weight of the body appeared to be taken off the forelegs altogether for a moment till the next dig was made. This was repeated three times, and it could not be seen whether any bending of the proboscis took place. After the third dig the proboscis remained buried in the skin for about its distal third and the fly stood absolutely motionless while sucking. The proboscis was coloured red by the inflowing blood, and the fly's abdomen gradually swelled until the proboscis was withdrawn. The whole act of feeding occupied 76 seconds, at the end of which time the fly's abdomen was fully distended. He remained motionless for over four minutes after feeding and was then removed.

The procedure of the second fly was similar, but he took 16 probing motions before he began to suck, and the act of feeding occupied 5 minutes and 20 seconds. The proboscis was buried for about half its extent, and was more gradually withdrawn than in the case of the other flies. During the probing movements there was noticed to be some bending of the proboscis. Immediately after feeding he cleaned his proboscis in the manner noted for the first fly.

X.—THE FOOD OF *GLOSSINA MORSITANS*.*

Five hundred flies, freshly caught in the Proclaimed or Sleeping Sickness Area of Nyasaland, were killed by chloroform and the gut of each was roughly dissected out, smeared on a slide, fixed by osmic vapour and alcohol, and subsequently stained by Giemsa. The flies were all caught in the bush, away from the paths, the fly-boys proceeding in single file and catching the flies with gauze nets as they circled round or settled on the boys or the grass.

The proportion of male flies to females caught was roughly 2 to 1. But only 30 females were used in the present experiment, the majority being sent to the breeding-station at Chunzi.

Of the 500 flies examined, 288, or 57.6 per cent., were found to contain mammalian blood in a recognisable state. No measurements were made of corpuscles, which in most cases were much altered by the digestive processes, but the small type of cell appeared to predominate, such as occurs in the hartebeeste, waterbuck and other antelope.

In only three cases were nucleated red corpuscles found, and in two of these there was only a small proportion of nucleated blood mixed with a large amount of mammalian. In the third case the blood was all nucleated. Thus, of those flies which contained recognisable blood, only 1.0 per cent. contained nucleated blood. From measurements it seems highly probable that in all three cases the blood was avian, not reptilian. The average length of corpuscles and nuclei of blood from several different reptiles was measured and found to be, corpuscles 15 microns, nucleus 5.9 microns; while the blood of several different birds gave as the average, corpuscles 11.8 microns, nucleus 4.6 microns. In the three cases under consideration the average of the corpuscles was 10.5, 10.0, and 10.0 microns respectively, and that of the nuclei 4.7, 4.8, and 4.4 microns. Probably the size of the nucleus is the better guide than that of the whole corpuscle, as being less altered by digestion.

In no case was vegetable matter noted in the intestinal contents.

Trypanosomes were found in 14 flies—2.8 per cent.—but many of the smears were so thick and so much obscured by the fat-body and other structures of the fly, that probably trypanosomes were present in other cases.

Of the 30 female flies examined, 13, or 43.3 per cent., contained mammalian blood, and there was nothing to suggest that they differed in their feeding habits from the males.

From experiments with flies in the laboratory, it was found that blood is recognisable in stained specimens for two or even three days after a feed, but not beyond the third day. Hence it may be inferred that, roughly, half the flies examined had fed within, at most, three days of their capture, and that therefore the flies feed naturally at least once every six days.

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 41.

Conclusions.

1. The food of *Glossina morsitans* consists mainly of mammalian blood (99 per cent.), chiefly from species of antelope, and what appeared to be avian blood (1 per cent.).
2. There is no difference in the feeding habits of males and females.
3. Probably the flies feed once in five or six days.

XI.—GLOSSINA BREVIPALPIS AS A CARRIER OF TRYPANOSOME DISEASE IN NYASALAND.*

Glossina brevipalpis (Newstead) is found at many spots along the west shore of Lake Nyasa. The nearest point to the Commission's camp at Kasu where they were at all common was at the mouth of the Lingadzi River (lat. 13° 37' S., long. 34° 19' E.). This was 50 miles away, but with the aid of a motor-cycle specimens of these flies were brought up to the camp.

It is proposed here to give a short account of the habits of this tsetse fly, the result of the dissection of the flies and the infectivity of the wild flies. The result of various transmission experiments with *Glossina brevipalpis* will be given later.

One of the members of the Commission camped on the Lake-shore from April 26 to May 10, 1913, to superintend the catching of these flies and sending them to the camp. He has supplied the following account of their habits.

Habits of Glossina brevipalpis.—*Glossina brevipalpis* is found frequenting the roads in a small area of country around Markho village on the west shore of Lake Nyasa, at the mouth of the Lingadzi River. This tract of country, which may be roughly estimated at about five square miles in extent, is broken up by swampy hollows and streams forming the delta of the Lingadzi. It is covered with palm forest and a dense undergrowth of high grass and bush, and is traversed by two main roads, one between Markho and Lingadzi estate running east and west, and another running parallel to the Lake-shore and about one mile to the west of it. The roads are hoed tracks about three yards broad, cut through the palm forest and walled in by high grass and almost impenetrable bush.

Glossina brevipalpis is crepuscular in its habits and quite unlike other Nyasaland tsetse flies. During the daytime, from dawn till about an hour before dark, one may pass along the roads or wander in the surrounding jungle without encountering a single fly of this

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 20.

species. But as evening approaches, odd flies suddenly appear sitting motionless in the middle of the hard trodden path all the way along the road between Markho and Kasache, a distance of two miles, and for about two or three miles up the Lingadzi road. They do not follow or settle upon passers-by like other tsetse flies, and they would pay no attention to a dog which was repeatedly walked through their haunts in the evening. In the dim light they are difficult to see, and resemble little bits of earth on the path, but the searcher's attention is attracted by the sound of them buzzing up as they are disturbed by his footsteps. They immediately settle on the path again, and are easily caught, for if missed by the first stroke of the net they at once resettle near the same spot. The hard trodden surface of the path seems to have an irresistible attraction for them. They do not move about in search of food or chase each other, but remain motionless for several minutes, and when they move it is only to fly up and immediately settle again in the middle of the path. They were never seen on the roads at dawn, as the mornings at this time of the year were invariably cold and misty.

Out of the 500 flies caught and examined on the spot, all were males.

Some of them were found on dissection to contain mammalian blood. Game, such as buffalo and several species of antelope, is common in the district.

Flies kept in captivity remain dormant on the sides of their cage during the day. At night they are very active and buzz incessantly in their efforts to escape. If the side of the cage be applied to the skin of a goat or a dog they will feed with avidity at any time of the day or night.

Food of G. brevipalpis.—A few wild flies were dissected, in order to ascertain their natural food. In 50 flies, seven contained recognisable blood. This, in six cases, was antelope blood, in the seventh probably human.

Proportion of the Sexes.—It is curious the great preponderance of males over females. It seems to be a habit of the former to frequent paths in the evening, while the females presumably hide in the thick jungle. The same thing obtained to a lesser extent with *Glossina morsitans*. Among all the *Glossina brevipalpis* dissected at the laboratory, amounting to several hundreds, only four females were found.

DISSECTION OF WILD *GLOSSINA BREVIPALPIS* TO ASCERTAIN WITH WHAT SPECIES OF TRYPANOSOMES, IF ANY, THEY ARE INFECTED.

Four hundred and ninety-six wild flies were examined: 44 of these were positive and 452 negative. The following table gives the result of the dissection of the positive flies:—

Table VII.

Date.	No. of fly.	Labial cavity.	Hypopharynx.	Pro-ventriculus.	Crop.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands
1912.									
June 14	...	1	—	—			+		—
„ 14	...	2	—	—	—	—	++	++	
„ 14	...	3	+			+	++		
„ 14	...	4	—	—	++	++	++		
„ 28	...	5	—	—			+		—
„ 28	...	6	—	—		+	+		
„ 28	...	7	+			++	++		
„ 28	...	8	—	—		++	++		
„ 28	...	9	—	—		++	++		
Sept. 12	...	10	—	—	++	++	++		
1913.									
Mar. 5	...	11	+	++		++	++	++	—
„ 18	...	12	—	—			++		—
„ 18	...	13	—	—		++	++	++	—
„ 21	...	14	—	+	—	—	+		—
Apr. 4	...	15	—	—		+	+	+	—
„ 22	...	16	—	—			+		
May 22	...	17					+		
„ 31	...	18	+			++	++		
„ 31	...	19	—	—			+		—
June 2	...	20	—	—			+		—
„ 2	...	21	+			+	+		—
„ 5	...	22	++	++		++	++	++	
„ 9	...	23	—	—			+		
„ 12	...	24	++	++	++	++	++	++	—
„ 23	...	25	—	—		++	++	++	++
„ 24	...	26	++	++		++	++	++	
„ 25	...	27	—	—			+		—
„ 26	...	28	—	—			+		—
„ 26	...	29				+	+		—
July 1	...	30	++	++		++	++	++	
„ 5	...	31	—	—			+		—
„ 11	...	32	—	—			+		—
„ 14	...	33	++	—		++	++		—
„ 14	...	34	—	—			+		—
„ 14	...	35	++	—		++	++		—
„ 16	...	36	—	—			+		—
„ 16	...	37	+	—		++	++		—
„ 16	...	38	++	+		++	++		—
„ 24	...	39	—	—			+		—
„ 24	...	40	+				+		—
„ 24	...	41	—	—			+		—
„ 24	...	42	—	—			+		—
„ 30	...	43	+	—			+		—
„ 30	...	44	—	—			+		

Among the 44 flies which contained flagellates, it was possible to make a more or less correct guess at the species in 19. These are *Trypanosoma brucei* vel *rhodesiense* 1, *Trypanosoma pecorum* 9, *Trypanosoma simiae* 1, and *Trypanosoma grayi* 8. In 10 flies the

C.—TRYPANOSOME DISEASE OF MAN AND DOMESTIC ANIMALS IN NYASALAND.

INTRODUCTORY.

In this section the various trypanosomes causing disease in man and domestic animals in Nyasaland are described.

It may be well at this point to indicate briefly the various species of pathogenic trypanosomes found in Africa. These may be classified into three groups:—

A GROUP.—*Trypanosoma brucei* Group:—

1. *T. brucei*.
2. *T. gambiense*.
3. *T. evansi*.
4. *T. equiperdum*.

B GROUP.—*T. pecorum* Group:—

1. *T. pecorum*.
2. *T. simia*.

C GROUP.—*T. vivax* Group:—

1. *T. vivax*.
2. *T. capræ*.
3. *T. uniforme*.

These constitute all the important disease-producing trypanosomes of Africa. With the exception of *T. evansi* and *T. equiperdum* they are carried from the sick to the healthy by tsetse flies. It may be convenient at this point to give a short description of each of them.

Each group is distinguished by certain characters. For example, in Group A, the *T. brucei* Group, the trypanosomes are all more or less polymorphic, varying in shape and size from short and stumpy forms to long and slender. *T. brucei* and *T. gambiense* also develop in the same way in the tsetse fly. At first the development takes place in the intestinal tract, afterwards the parasites pass forward through the labial cavity and invade the salivary glands by way of the hypopharynx.*

In Group B, the *T. pecorum* Group, the trypanosomes are monomorphic and develop in the intestinal tract, labial cavity and hypopharynx of the fly, but do not invade the salivary glands.

In Group C, the *T. vivax* Group, the trypanosomes are very active, monomorphic, and develop in the labial cavity and hypopharynx of the fly, but not in the intestinal tract or salivary glands.

* "Notes on the Life History of *T. gambiense*," etc. By Muriel Robertson, M.A. "Philosophical Transactions" of the Royal Society, B, vol. 203, p. 161, and Reports of the Sleeping Sickness Commission of the Royal Society, No. XIII, p. 119.

GROUP A.

1. *Trypanosoma brucei* (Plimmer and Bradford, 1899).

Synonyms: *T. rhodesiense* (Stephens and Fantham, 1910); *T. ugandæ* (Stephens and Blacklock, 1913).—This was the first species of pathogenic trypanosome discovered in Africa. It is the cause of the so-called "Nyasaland sleeping sickness" in man. In Zululand the disease in animals is called "Nagana" by the natives, and is now commonly known by this name.

Distribution.—It is widely distributed in Africa, extending from the Sudan to Zululand.

The districts in Africa where cases of "Nyasaland sleeping sickness," or "*T. brucei* disease in man," have been found are Northern Rhodesia, where cases have been found in the Luangwa Valley, in the Serenje District to the south-east of Lake Bangweolo, in the Mpika District, in the Ndola District, and in the Luana Valley. In Southern Rhodesia in the Sebungwe District. In Nyasaland in the Marimba, Central Angoniland, South Nyasa, and Upper Shire Districts. In German and Portuguese East Africa in the valley of the Rovuma River. (See Map 2, p. 38.)

In the opinion of the Commission it is probable that cases will be found to occur wherever *Glossina morsitans* and wild game abound.

Morphology.—*T. brucei* is very various in shape, some forms being short and stumpy with little or no free flagellum, and others long and slender with a well-developed free flagellum.

Susceptibility of animals.—Animals of many different species are attacked by this trypanosome. Among these may be mentioned man, horses, mules, donkeys, oxen, goats, sheep, dogs, rabbits, guinea-pigs, rats, and mice.

Reservoir.—This parasite is found in the blood of wild animals throughout a large part of Africa.

Carrier.—The carrier of *T. brucei* in Nyasaland is the common tsetse fly, *Glossina morsitans*. The development of *T. brucei* in *G. morsitans* takes place at first in the intestinal tract of the fly. After some 20 or 30 days the trypanosomes pass forward through the labial cavity of the proboscis, and, probably, by way of the hypopharynx, into the salivary glands, where they multiply to an extraordinary extent. The trypanosomes undergoing development in the intestinal tract are not infective. It is only when the trypanosomes reach the salivary glands that they assume the "blood form" and become infective.

2. *Trypanosoma gambiense* (Dutton, 1902).

This is the parasite which gives rise to the "Uganda sleeping sickness."

Distribution.—It has a wide distribution in Africa between 16° N. lat. and 14° S. lat.

Morphology.—It is very similar to *T. brucei* in shape. It is doubtful if it is possible in every case to distinguish *T. brucei* from *T. gambiense* by morphology alone.

Susceptibility of animals.—This disease is, as a rule, fatal to man. It is usually chronic and may last for years. The natives, however, who are often neglected by their friends, frequently died in Uganda in as short a time as five or six months. In the French Congo it has been stated that as many as 17 per cent. were still alive after six years. Among Europeans, recoveries take place under treatment. *T. gambiense* differs in a marked way from *T. brucei* in its behaviour to animals other than man. *T. brucei* infects most animals—horses, monkeys, dogs, and rats—readily, and kills them off quickly. On the other hand, it is a matter of difficulty to infect animals with *T. gambiense*. It would almost appear as if *T. gambiense* had become adapted to live in human blood and not in the blood of the lower animals.

Reservoir.—The native himself is probably the most common reservoir of this virus, but wild monkeys, antelope, and domestic cattle have also been found infected, and the antelope are undoubtedly keeping up the infectivity of the fly at the present time on the shores of Victoria Nyanza. The natives were removed from these unhealthy areas some years ago, but the tsetse flies (*Glossina palpalis*) still remain infective.

Carrier.—The "Uganda sleeping sickness" is carried from the sick to the healthy by means of the tsetse fly (*Glossina palpalis*). The development of *T. gambiense* in *Glossina palpalis* is similar to that of *T. brucei* in *Glossina morsitans*.

3. *Trypanosoma evansi* (Steel, 1885).

This is a trypanosome disease of North Africa and India, where it is known as surra. It principally attacks camels and horses. It has probably spread along the camel routes from Africa into India.

Distribution.—This disease is known in the Sudan and neighbouring parts of North Africa.

Morphology.—Belongs to the *T. brucei* group.

Reservoir.—It is probably spread from one infected animal to another. It is, however, possible that the native Indian buffalo may act as reservoir.

Carrier.—It is thought at present that it is carried mechanically from sick animals to healthy by means of the large biting flies, the tabanids or horse flies. It is believed that no special development of the parasite takes place in these flies, but that they merely carry the virus from one animal to another in the same way that a surgeon carries vaccine from one child to another. But it must be confessed that this is still a matter of speculation as little serious research has been done on the subject.

GROUP B.

1. *Trypanosoma pecorum*.*

This parasite gives rise to a fatal disease among cattle, goats, sheep, and dogs, and is probably the most important trypanosome disease in Africa among the bovines.

* "Proceedings" of the Royal Society, B, vol. 82, 1910, p. 468, and Reports of the Sleeping Sickness Commission of the Royal Society, No. XI., p. 128.

Distribution.—It is widely distributed in Africa, occurring in Uganda, Northern Rhodesia, Nyasaland, Zanzibar, Zululand, etc.

Morphology.—It is one of the smaller types and does not possess a free flagellum.

Animals susceptible.—Horses, donkeys, oxen, goats, sheep, dogs, etc., are attacked.

Reservoir.—Common in the blood of wild game in many parts of Africa.

Carrier.—The tsetse fly *Glossina morsitans* in Nyasaland, and probably *Glossina palpalis* in Uganda, but there is some circumstantial evidence available that when this disease has once been introduced into a herd by the tsetse fly it may spread from one animal to another by means of other biting flies, as *Tabanidæ* and *Hæmatopota*. The development in *Glossina morsitans*, as already mentioned, takes place in the intestinal tract, the labial cavity, and the hypopharynx.

2. *Trypanosoma simiæ*.*

Synonym.—*T. ignotum* (Kingham and Yorke, 1912).

Gives rise to a rapidly fatal disease in the monkey and the domestic pig. Frequently a healthy pig will die in four to six days if bitten by a tsetse fly infected by this trypanosome.

Distribution.—Only found up to the present in N.E. Rhodesia and Nyasaland.

Morphology.—Closely resembles *T. pecorum* except that it is longer.

Animals susceptible.—An interesting feature in this trypanosome is the virulence it displays towards monkeys and the domestic pig, killing these animals in an incredibly short period of time, whereas it is harmless to oxen, antelope, dogs, and the smaller experimental animals, such as the rabbit, guinea-pig, and rat. Curiously enough, this trypanosome also infects goats and sheep, although cattle and antelope escape.

Reservoir.—The warthog seems to be the only reservoir of this parasite among wild animals. It will probably be also found in the bush-pig, but the Commission failed to obtain any of these animals for examination.

Carrier.—*Glossina morsitans*. The development of this parasite in *Glossina morsitans* is identical with that of *T. pecorum*.

GROUP C.

1. *Trypanosoma vivax* (Ziemann, 1905).

Horses, oxen, and goats seem to be the animals chiefly affected by this disease.

Distribution.—Wide distribution in Africa—Gambia, Senegal, Dahomey, Northern Nigeria, Cameroons, Uganda, German East Africa, and the Sudan. The Commission did not find it in Nyasaland, where it seems to be replaced by *T. capræ*.

* "Proceedings" of the Royal Society, B, vol. 85, 1912, p. 477, and Reports of the Sleeping Sickness Commission of the Royal Society, No. XV., p. 11.

Morphology.—A characteristic of this species is its extreme rapidity of motion when alive. Has a large micronucleus and clear protoplasm.

Susceptibility of animals.—One peculiarity of this trypanosome is that it is harmless to monkeys, dogs, rabbits, guinea-pigs, and rats.

Reservoir.—The wild game.

Carrier.—*Glossina palpalis* in Uganda. The development takes place only in the labial cavity and the hypopharynx.

2. *Trypanosoma capræ* (Kleine, 1911).*

Similar to *T. vivax* in its action on animals.

Distribution.—It has only been reported from German East Africa and Nyasaland.

Morphology.—Very similar to *T. vivax*, but stouter in build.

Susceptibility of animals.—Goats and sheep are attacked.

Reservoir.—The wild game.

Carrier.—*Glossina morsitans*.

3. *Trypanosoma uniforme*.†

Distribution.—Only found up to the present in Uganda.

Morphology.—Similar to *T. vivax*, but smaller.

Susceptibility of animals.—Cattle, goats, and sheep are susceptible to this species of trypanosome, but not monkeys, dogs, or smaller experimental animals.

Reservoir.—Found in antelope in Uganda.

Carrier.—*Glossina palpalis*.

These, then, are the principal trypanosome diseases of man and domestic animals in Africa.

Four species were found in Nyasaland—*Trypanosoma brucei* vel *rhodesiense*, *Trypanosoma pecorum*, *Trypanosoma simiæ*, and *Trypanosoma capræ*.

The most important of these is *Trypanosoma brucei* vel *rhodesiense*, as this, in the opinion of the Commission, is the species which gives rise to disease in man in Nyasaland. This raises the question of the identity or non-identity of *Trypanosoma brucei* (Plimmer and Bradford) and *Trypanosoma rhodesiense* (Stephens and Fantham). This is a most important question, and, with our present knowledge, one impossible to answer at the present time with absolute certainty.

* "Trypanosomenstudien," von Dr. F. K. Kleine and Dr. M. Taute, 1911.

† "Proceedings" of the Royal Society, B, vol. 83, 1911, p. 176, and Reports of the Sleeping Sickness Commission of the Royal Society, No. XI., p. 160.

ON THE IDENTITY OR NON-IDENTITY OF *T. BRUCEI* VEL
RHODESIENSE.

If *Trypanosoma brucei* and *Trypanosoma rhodesiense* are identical, then we must expect to find cases of "Nyasaland sleeping sickness" cropping up in all parts of Africa where *Glossina morsitans* and *Trypanosoma brucei* occur. If, on the other hand, they are separate species, then cases of this disease would only be expected to occur where *Trypanosoma rhodesiense* is found in addition to *Trypanosoma brucei*. In the past, *Trypanosoma brucei* has always been regarded as harmless to man; and certainly no harm seemed to come to those who lived or travelled in fly-country.

It must be explained at the outset that, up to about the year 1908, it was believed that *Trypanosoma gambiense* was the only trypanosome capable of setting up disease in man. It must also be clearly kept in mind that up to that time no case of trypanosome disease in man had ever been found in an exclusively *Glossina morsitans* or "Nagana" area. All cases of sleeping sickness up to then had been found in districts where *Glossina palpalis* was present. Since the year 1908, however, cases have been discovered in Northern and Southern Rhodesia, in Nyasaland, and in Portuguese East Africa, where there are no *Glossina palpalis*, but where only *Glossina morsitans* is found.

At first it was supposed that this was a spread of "Uganda sleeping sickness," or "*Trypanosoma gambiense* disease," from Lake Tanganyika along the trade route through the Luangwa Valley into Nyasaland. And in truth this explanation seems very natural. But in the Liverpool School of Tropical Medicine it was discovered by Stephens and Fantham that the trypanosomes causing the "Nyasaland sleeping sickness" differed in some ways from *Trypanosoma gambiense*, and it was therefore called *Trypanosoma rhodesiense*, and the disease declared to be a new disease.

In 1912, the Commission, in further studying this question, also came to the conclusion that "Nyasaland sleeping sickness" and "Uganda sleeping sickness" were separate diseases. In other words, that *Trypanosoma gambiense* and *Trypanosoma rhodesiense* were different species.

The Commission was soon struck, however, by the fact that although the fly-country of Nyasaland extends almost uninterruptedly into the Zululand fly-country, no *Trypanosoma brucei*—the common trypanosome of wild game in Africa—could be found. Everywhere, in the wild tsetse flies and in the wild game, it was always *Trypanosoma rhodesiense*. It then began to dawn on the mind of the Commission that perhaps *Trypanosoma rhodesiense* was in reality the old *Trypanosoma brucei* masquerading under a new name. At once, by the kindness of Sir Arnold Theiler, K.C.M.G., a strain of *Trypanosoma brucei* from Zululand was procured for purposes of comparison; and it may be well at this point to give the arguments in favour of the theory held by the Commission that *Trypanosoma brucei* and *Trypanosoma rhodesiense* are one and the same species.

In trying to separate species of trypanosomes from one another, the following points may be considered:—

1. Are they alike in size and shape? Morphology.
2. Have they the same habits and actions when alive?
3. Do the two trypanosomes infect the same animals and kill them in the same time?
4. Have they the same carrier? and does their growth or development in the carrier follow the same course?
5. Cross-inoculation experiments.—If an animal has recovered from an attack of disease set up by one of the two trypanosomes, is it immune or non-immune to infection by the other?
6. Experiments on the effect of various sera.
7. Geographical distribution.

1. In regard to the first point—and a most important point—namely, the morphology, if the coloured plates and black-and-white drawings of *Trypanosoma brucei*, Zululand,* and *Trypanosoma rhodesiense*,† Nyasaland, are compared, it will be evident that in size and shape these two trypanosomes are identical.

2. They are also alike in habits and actions when alive.

3. They have the same action on cattle, goats, sheep, monkeys, dogs, rabbits, guinea-pigs, and white rats. *Trypanosoma rhodesiense*, Nyasaland, is also fatal to man, but as it is impracticable to obtain man for experiment, it is impossible to say whether *Trypanosoma brucei*, Zululand, would or would not infect him. In the opinion of the Commission, if the experiment was repeated sufficiently often, it would succeed in infecting man. On the other hand, it will be urged that Dr. Taute, in Portuguese East Africa, injected under his skin blood from an animal infected with what he considered to be *Trypanosoma brucei*, and also allowed tsetse flies, infected with the same trypanosome, to feed on him. Both experiments were negative. It is a matter for some scientific regret that these experiments were not successful—though we can ill spare our bold and somewhat rash colleague—as then the question would have been answered. As it is, these negative experiments prove nothing. It may be that only one man in a thousand would become infected in this way.

There is also the argument that men have been exposed to *Trypanosoma brucei* for many years, and no case of *Trypanosoma brucei* disease has occurred. To this it may be answered that it is only within recent years that microscopical examination of the blood of such cases has been made.

Many cases of death are reported to have occurred among hunters and explorers, and these were often put down to “malaria”; but as there was no blood examination, it is quite possible that some of these cases might have been due to infection by *Trypanosoma brucei*. It is doubtful if man ever dies of uncomplicated malaria.

* “Proceedings” of the Royal Society, B, vol. 87, 1914. Plates XXII. and XXIII., p. 510.

† *Ibid*, B, vol. 85, 1912. Plates 11 and 12, p. 433.

Take the case of kala-azar: this widespread disease, which is common in India, and, indeed, in many parts of the world, remained undiscovered until a few years ago. This was about the year 1900. Up to that time, all these thousands of cases had been returned as "chronic malaria." The reason why these cases of kala-azar were not properly diagnosed was also due to the fact that up to 1900 few microscopic examinations of the organs of this disease were made. The discovery of these minute parasites also often depends on the introduction of some new mode of staining.

Or take the case of Malta fever: up to the year 1886, when its cause was discovered, all cases of this well-marked and widely-distributed fever were returned as "remittent fever," or "enteric fever," although its symptoms and *post-mortem* features bear no resemblance to either of these diseases.

Typhus fever and enteric fever is another case in point.

Now, just as in the case of kala-azar, Malta fever, and enteric fever, so, in the opinion of the Commission, cases of *Trypanosoma brucei* disease may have occurred in Zululand, Nyasaland, and other parts of Africa where wild game and *Glossina morsitans* are found, without attracting attention.

It is to be regretted that this important point must still remain in the domain of speculation and not rest on the firmer foundation of fact and experiment; but, it is repeated, nothing can be done at present to settle this question satisfactorily, since man may not be used as an experimental animal.

It will be found difficult, or even impossible, to arrive at a certain decision in regard to the identity of *Trypanosoma brucei* and *Trypanosoma rhodesiense*. Many apparently cogent arguments can be advanced against the theory.

One of the strongest witnesses on the opposing side is Dr. Murray, late of the Mvera Mission of the Dutch Reformed Church, Nyasaland. He is of opinion that no cases of sleeping sickness could have occurred in the Proclaimed Area, Nyasaland, before the year 1908. Dr. Murray was at that time medical missionary at the mission station. He states that he saw hundreds of cases at his dispensary before 1908, and he believes that if there had been cases of sleeping sickness before that time he must have noticed the fact. He also is of opinion that the cases which have occurred at Hartley and around Salisbury in Southern Rhodesia were, without doubt, infected through natives from Nyasaland, or from the Luangwa, who had gone south in search of work, having easily evaded the regulations of the Sleeping Sickness Area against their leaving.

But, on the other hand, it may be argued that the Nyasaland native suffering from sleeping sickness is more apt to remain in his own hut than ask for medical advice in a mission dispensary. If a native has something tangibly wrong with him—a broken arm, a wound, an ulcer—he is much more likely to seek advice than when he is suffering from some fever. And then, again, this disease makes such rapid progress that in a short time the native is unable to travel.

There are comparatively few cases of "Nyasaland sleeping sickness" in the Proclaimed Area. Since 1908, up to March, 1914, only some 150 cases have been found, and that after much searching.

As there must be 15,000 to 20,000 natives in this district, it seems quite probable that Dr. Murray never had a case at his dispensary. Even after it was known that this disease existed in his district, the Commission is not aware that he found any cases.

4. *Trypanosoma brucei* and *Trypanosoma rhodesiense* have the same carrier—*Glossina morsitans*—and their development in this fly proceeds in the same way.

5. No satisfactory evidence against these two trypanosomes being the same species has been brought out by cross-inoculation experiments. The difficulty in regard to cross-inoculation experiments is, that a strain of a trypanosome may be so weakened in virulence by some means that it fails to immunise an animal against a more powerful strain.

6. Experiments with human and other animal sera have also been unsatisfactory in establishing the identity or non-identity of these two trypanosomes. It would appear to be natural that a strain of a trypanosome, which had been living in human blood for some time, would behave differently to human serum than one which had been living in the blood of antelope.

7. In regard to the geographical distribution of "Nyasaland sleeping sickness," if Dr. Murray's theory is correct, that all these outlying outbreaks of trypanosome disease in man are due to infection from Lake Tanganyika, then the geographical distribution of the disease will not help in establishing the identity or non-identity of these two trypanosomes.

Dr. Murray's theory is that "Uganda sleeping sickness," or "*Trypanosoma gambiense* disease," has spread into Nyasaland from Lake Tanganyika. If it can be proved that *Trypanosoma gambiense* and *Trypanosoma brucei* vel *rhodesiense* are separate species, then his theory falls to the ground.

The Commission thinks that, in addition to the morphology and action on animals, the geographical distribution of "Nyasaland sleeping sickness," or "*Trypanosoma brucei* disease," is the only way—experiment on man being impracticable—of showing that *Trypanosoma brucei* and *Trypanosoma rhodesiense* are one and the same.

If, in the future, cases of this disease are found in widely-separated places, where *Glossina morsitans* and wild game abound, then it would be a strong argument that this form of sleeping sickness is caused by *Trypanosoma brucei*. Are there sufficient facts at present to enable an opinion to be formed? Hardly, the Commission is afraid; but they are multiplying. Take, for example, the discovery of cases in the Sebungwe District in Southern Rhodesia. Even Dr. Murray will hardly be able to say that this is due to infection from Nyasaland. Then the cases in German and Portuguese East Africa are difficult of explanation from his point of view. Further, cases have just been found in Nyasaland, in the Marimba District, north of the Proclaimed Area, in the South Nyasa and Upper Shiré Districts, south of the Proclaimed Area, showing that the disease is not confined to this district through which the trade-route from Rhodesia passes.

The disease has been found in several districts in Northern and Southern Rhodesia, in Nyasaland, and in German and Portuguese East Africa (see Map 2, facing). In all these widely-separated places *Glossina morsitans* is the tsetse fly present. In our opinion, then, evidence is accumulating from the geographical distribution point of view, that this new kind of sleeping sickness is really caused by *Trypanosoma brucei*, and that the name *Trypanosoma rhodesiense* must be dropped. In this Report, however, to avoid ambiguity, the double name *T. brucei vel rhodesiense* will be adopted.

The opinion of the Commission also is that the disease "Nyasaland sleeping sickness" has not been introduced into Nyasaland from Lake Tanganyika, but that it is an endemic disease of the country.

Before passing on to describe *Trypanosoma brucei vel rhodesiense* in detail, it may be well to describe the disease in man set up or caused by the entrance into his blood of this parasite.

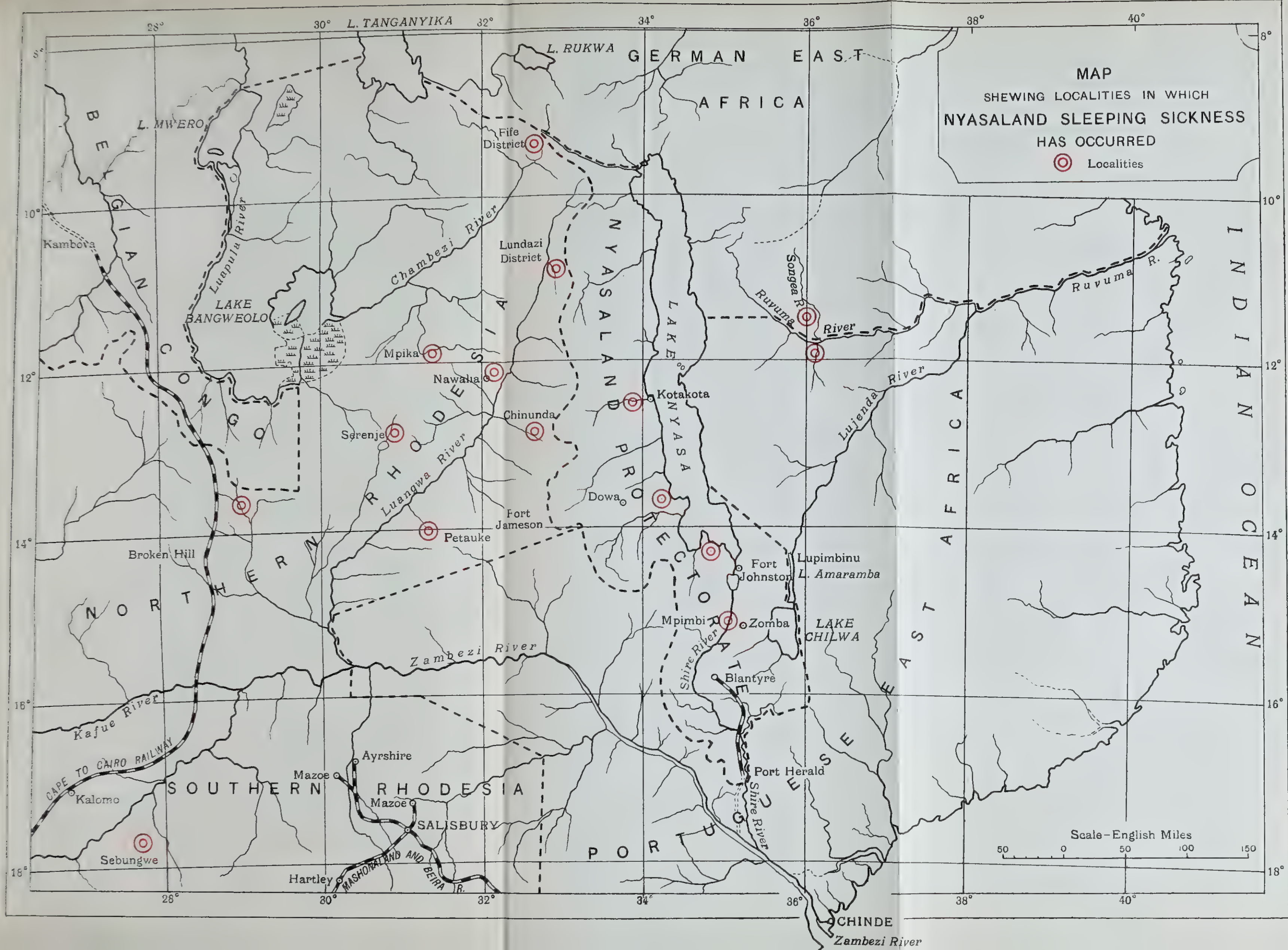
XII.—THE CLINICAL ASPECT OF HUMAN TRYPANOSOME DISEASE IN NYASALAND.

The following account is compiled from papers by Drs. Sanderson, Shircore, and others,* and from records of cases treated in the Proclaimed Area. The Commission had no opportunity of studying the disease in man, as no cases were brought up to the camp at Kasu.

History.—The first case was found in the West Nyasa District in 1908, and investigations were at once carried out in the northern districts and on the S.W. shore of Lake Nyasa. Sixty thousand persons were examined by means of gland palpation and blood smears from the sick, but no further cases were discovered. At the end of 1908 the late Captain Hardy, R.A.M.C., was found to be infected, and himself believed that he had incurred the infection in the bush country bordering Lake Nyasa. In 1909 and 1910, six more cases were diagnosed. Up to this time it was supposed that all these infections might have been contracted outside the Protectorate. But late in this year a European member of the Dutch Reformed Church, visiting Mvera Mission, contracted the disease in circumstances which made it certain that he had become infected in the neighbourhood. Fresh investigations were at once started, and an isolation camp was opened at Ngani in May, 1911. This was subsequently closed, owing to the reluctance of the natives to be treated there. Up to March, 1914, 178 cases have been recorded in all.

Etiology.—In all cases the trypanosome causing human disease in Nyasaland, *Trypanosoma brucei vel rhodesiense* has been found in the blood, and can usually be found in increasing numbers as the disease advances.

* Transactions of the Soc. of Trop. Med & Hyg., July, 1912, p. 295, and February, 1913, p. 131.





Sex.—Of 166 native cases, 111 were men and 55 women, so that the incidence among males is exactly double that among females. This is probably due to the fact that the men wander further afield and are more exposed to the bites of the tsetse flies.

Age.—The average age of 70 cases was 33·9 years. Few children have been found infected, and, with the exception of one infant of seven months, none under eight years of age. Of the males, the average age was 30·9 years, and of the females 41·35 years. The greatest age recorded was 74 years. The age-average of the females was brought up by three, out of the 20 recorded, who were 60 years old or more. This high proportion of aged women among the infected is probably related to the fact that the old women spend much time working in the gardens away from the villages. In fact, the age and sex incidence both appear to bear a direct relation to the extent to which persons are in the habit of leaving the village clearing. Thus, children are scarcely affected; women to half the extent only of men: and of the women a large proportion are old women accustomed to work in the gardens and carry in wood from the bush.

Season prevalence.—Analysis of the monthly incidence does not show any marked seasonal prevalence.

Incubation period.—Among natives it is rarely possible to obtain a reliable account of the circumstances attending the onset of the disease. In a few cases, however, a definite history was elicited of illness following a journey through the bush, usually for the purpose of visiting a neighbouring village. Seven such cases, in which the data appear to be reliable, give an average incubation period of eight days, which closely corresponds with that observed in experimental animals. It is certain that the incubation period is short. In three European cases it appeared to be from six to 14 days.

Duration.—The course of the trypanosome disease in Nyasaland differs markedly from that of sleeping sickness due to *Trypanosoma gambiense*, in being much more rapid, and the typical somnolent stage of the latter is very seldom seen. The onset is definite and severe. In all probability no authenticated case has recovered. In one case, that of a European, the disease lasted three years. But this is a doubtful case and is quite exceptional. The average duration of 37 cases, in which a reliable date of onset could be obtained, was 4·2 months. The longest duration was 14, and the shortest two months. The disease runs a more rapid course in children than in adults.

Symptoms.—The course of the disease, subsequent to the incubation period, may be divided into three stages:—

- (1) The initial fever.
- (2) An intermediate stage marked by progressive asthenia and impaired cerebration, during which the patient can still get about.
- (3) The terminal stage, with marked mental change, ending in coma and death.

(1) *The initial fever.*—In each of the Europeans there was marked local reaction to the bite of *Glossina morsitans*; in each case in the sub-occipital region, a common site of attack in Europeans. Only rarely has a similar history of local reaction been obtained from natives. Such local reaction would probably be considered as a common boil and pass unnoticed. There is probably an initial pyrexial attack in all cases, the fever being remittent in type, and ranging from 100° F. to 104° F., or more. Headache and pains in the limbs and body commonly accompany the initial pyrexia; occasionally a rigor. With high pyrexia there is sometimes delirium; but apart from this no disturbance of mental functions at this stage. In some cases the onset is marked by abdominal pains and diarrhoea; rarely vomiting. Or there may be obstinate constipation. Œdema of the feet sometimes occurs in this stage. Trypanosomes are scanty in the blood, one or two in a whole preparation. The initial stage lasts from three to four weeks, passing into the intermediate stage.

(2) *The intermediate stage.*—Is the one in which most native patients come under observation. In this stage the patient is able to keep up and about, and may, for a time, appear to improve and feel in fairly good health. But, eventually, emaciation, weakness and anæmia become progressive. The pyrexia is now intermittent in type, and may show marked periodicity. This periodicity is lost in the later stages, when there is a regular daily swinging intermittent fever, rarely rising above 101° F. The pulse and respiration rate show no constant ratio to the temperature; but the ratio of pulse to respiration rate is almost constantly four to one. The pulse is commonly regular, but of low tension. In most cases there is præcordial pulsation in the mitral area, and often marked pulsation along the course of the carotids, especially in robust subjects. Acute myocardial degeneration leading to syncope does not occur. Anæmia is marked. The red corpuscles number 3,200,000 to 3,500,000 per c.cm. The hæmoglobin averages 35 to 40 per cent., and may be as low as 25 per cent. The total leucocytes vary from 7,000 to 12,000 per c.cm., and there is an early relative lymphocytosis, which disappears towards the end of the disease. Although the tongue is furred, the lips dry, and the breath foul, the appetite is generally well maintained. But occasionally vomiting, abdominal pain and diarrhoea occur. The muscles waste. Tremors of the hands and tongue were noted in 70 per cent. of the cases; shuffling or slow gait in 25 per cent.; and altered speech, thick or high-pitched, in 20 per cent. Pain in the legs is of frequent occurrence, and is probably due to neuritis, the deep reflexes becoming later often diminished or abolished. At first the patients appear highly-strung and nervous, then cerebation is impaired. Later, intelligence and memory are affected. In rare cases there is a short somnolent stage. Local transient œdemas, and œdema of the face and feet are common. The latter symptom was noted in 77·5 per cent. of the cases. Enlargement of lymphatic glands was noted in 87·5 per cent. of the cases, that of the epitrochlear glands being far more constant than of the cervical, and almost invariably present. Interstitial keratitis occurred in 15 per cent. of the cases. Independent of this, there is not uncommonly complaint of dimness of vision. The skin is commonly harsh and dry, and desquamation was noted in 20 per cent. of cases,

usually on the upper part of the trunk and base of the neck. Circinate erythema, a papular rash, and morbilliform rashes have also been noted. Trypanosomes are found in the blood in increasing numbers, and, in the last month of the disease, may number six to eight or more to the microscopic field. This stage lasts one to four or five months, or more.

(3) In the final stage the patient is unable to sit up, and there is marked mental deterioration, proceeding to coma and death. The temperature is still intermittent, or may be sub-normal, and the pulse and respiration rates are sub-normal, diminishing until death. Trypanosomes are more numerous in the blood than in the preceding stages, and dividing forms may be seen. In some cases, however, after coma has set in, they can be no longer found in the blood. The duration of this stage is from a few days to a month.

Treatment.—No method of treatment employed has had more than a very temporary beneficial effect. Atoxyl and soamin are the drugs that appear to have had most effect in diminishing the number of trypanosomes in the blood. Other drugs that have been employed are salvarsan, arsamin, and tartar emetic. In regard to treatment by vaccines or serum, it may be said that, up to the present, no definite success has been obtained either in man or animals.

XIII.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME — *TRYPANOSOMA BRUCEI* VEL *RHODESIENSE* — CAUSING DISEASE IN MAN IN NYASALAND. THE HUMAN STRAIN. STRAIN I.

This paper has already been published in the "Proceedings" of the Royal Society, B, vol. 85, 1912, p. 423, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 1.

It describes a single strain of this trypanosome taken from a native woman suffering from "Kaodzera," the Nyasaland form of sleeping sickness. When the paper was written, it was not suspected that *Trypanosoma brucei* and *Trypanosoma rhodesiense* were the same. The conclusions given at the end of the paper are:—

1. The trypanosome of the human trypanosome disease of Nyasaland is *T. rhodesiense* (Stephens and Fantham).
2. This is a distinct species, nearly related to *T. brucei* and *T. gambiense*, but more closely resembling the former than the latter.
3. From this it follows that the human trypanosome disease of Northern Rhodesia and Nyasaland is not the disease known as sleeping sickness in Uganda and the West Coast of Africa.
4. The native name "Kaodzera" might be used for this new disease to distinguish it from the older known sleeping sickness.

Two plates are given, one coloured and the other black and white, showing the various forms of this trypanosome, and especially the posterior-nucleated, short and stumpy forms.

The first conclusion would now be written: "The trypanosome causing disease in man in Nyasaland is *T. brucei* vel *rhodesiense*," and the fourth, "The name 'Nyasaland sleeping sickness,' or '*T. brucei* disease,' may be used to distinguish this form from 'Uganda sleeping sickness,' or '*T. gambiense* disease.'"

In the following papers several strains of this trypanosome are described. Ten strains from human sources, five from wild game, five from wild *Glossina morsitans*, one from a donkey infected at Mzimba in Mombera District, 100 miles north of Kasu, three from the Upper Shiré District, 100 miles south of Kasu, and, lastly, a strain from Zululand where *Trypanosoma brucei* was first discovered and described. These 25 strains, or races, are all believed by the Commission to belong to the same species—*Trypanosoma brucei*. This belief is not shared by the majority of workers on trypanosome diseases. It is held by most that mixed up with *Trypanosoma brucei* there is another species, *Trypanosoma rhodesiense*, which is alone capable of giving rise to disease in man.

In addition to the 25 strains, there are three others, called the Naturally-infected Dog Strain. This name was given on account of it having been recovered from the blood of three dogs found infected with trypanosome disease in the Sleeping Sickness Area. This strain is very different from the rest, and, if it had been found in the wild game and wild *Glossina morsitans*, would have deserved specific rank. But, as it was only found in the three dogs, it is considered to be an aberrant strain and placed in the *Trypanosoma brucei* species. As there are three strains of this Naturally-infected Dog Strain described, this brings up the total number of strains of this species dealt with to 28.

XIV.—THE HUMAN STRAIN. STRAINS II. TO V.

This paper has also been published in the "Proceedings" of the Royal Society, B, vol. 86, 1913, p. 285, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 31.

It describes four more strains—II. to V.—of this trypanosome taken from one European and three natives. At the end of the paper it is noted that evidence is accumulating that *Trypanosoma brucei* and *Trypanosoma rhodesiense* are identical.

XV.—THE HUMAN STRAIN. STRAINS VI. TO X.*

In former papers† five strains of this species of trypanosome, obtained from man, were described. In this, it is intended to describe another set of five.

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 190.

† "Proceedings" of the Royal Society, B, vol. 85, 1912, p. 423, and B, vol. 86, 1913, p. 285, and Reports of the Sleeping Sickness Commission, No. XV., pp. 1 and 31.

A small quantity of blood was taken from the sick native, brought up to the laboratory, and inoculated into a monkey or white rat.

Strains VII., VIII., and X. were directly inoculated into the rat from which the drawings and measurements were made; in Strains VI. and IX. a single monkey intervened. These five strains have, therefore, been obtained under fairly similar circumstances, and should prove useful for purposes of comparison. This is put in tabular form in Table IX, and the history of the previous five strains is also given, as this was omitted in the former papers. It is possible that the type of a strain may be changed by passage through different animals before it reaches the rat.

Table IX.—Showing the Passages through Animals between Man and the Rat whose trypanosomes are drawn and measured.

Strain.					Man.	Monkey.	Dog.	Rat.
I.	Mkanyanga	...	...	...				
II.	Eastwood	...	...	...	1st		2nd	3rd
III.	Chituluka	...	...	...	1st		2nd	3rd
IV.	Chipochola	...	...	...	1st		2nd	3rd
V.	Chibibi	...	...	...	1st		2nd	3rd
VI.	Manakumpara	...	...	...	1st	2nd		3rd
VII.	Yoramu	...	...	...	1st			2nd
VIII.	Mekka	...	...	...	1st			2nd
IX.	Mkanthama	...	...	...	1st	2nd		3rd
X.	Dongolosi	...	...	...	1st			2nd

From this table it will be seen that little time was lost in inoculating the rat, from which the trypanosomes were drawn and measured.

On comparing the curves from the ten strains, it cannot be said that the passage from dog to rat, or from monkey to rat, or direct from man to rat, has had any marked influence on the character of the curve. But in these cases, only a single monkey or dog intervened.

In the case of Strain I., Mkanyanga, the trypanosomes were taken at random from several species of animals.* Six hundred trypanosomes from four different rats were measured. The passages are as follows:—1, Man, Monkey, Rat 38; 2, Man, Dog, Rat, Rat, Rat 37; 3, Man, Monkey, Guinea-pig, Monkey, Rat 236; 4, Man, Monkey, Guinea-pig, Monkey, Rat 235.

Morphology of Strain VI., Manakumpara.

The following table gives the average length of this trypanosome as found in the white rat, 500 trypanosomes in all, and also the longest and shortest:—

* "Proc." Roy. Soc., B, vol. 85, 1912, p. 427, and Reports of the Sleeping Sickness Commission, No. XV., p. 6.

Table X.—Measurements of the Length of the Trypanosome of Strain VI., Manakumpara.

Date.	Expt. No.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
June 30	2239	Rat	Osmic acid	Giemsa.	21·4	27·0	18·0
" 30	2239	"	"	"	21·6	29·0	18·0
" 30	2239	"	"	"	22·1	27·0	20·0
July 1	2239	"	"	"	19·0	23·0	17·0
" 1	2239	"	"	"	18·6	23·0	15·0
" 1	2239	"	"	"	18·6	23·0	16·0
" 2	2239	"	"	"	19·8	22·0	17·0
" 2	2239	"	"	"	20·6	25·0	17·0
" 2	2239	"	"	"	19·3	21·0	17·0
" 3	2239	"	"	"	21·1	27·0	17·0
" 3	2239	"	"	"	21·8	26·0	17·0
" 3	2239	"	"	"	22·6	30·0	17·0
" 4	2239	"	"	"	23·0	26·0	17·0
" 4	2239	"	"	"	23·0	29·0	18·0
" 4	2239	"	"	"	23·0	28·0	19·0
" 5	2239	"	"	"	22·4	28·0	17·0
" 5	2239	"	"	"	21·8	30·0	17·0
" 5	2239	"	"	"	23·8	29·0	18·0
" 7	2239	"	"	"	23·5	28·0	21·0
" 7	2239	"	"	"	22·9	30·0	17·0
" 7	2239	"	"	"	24·5	32·0	19·0
" 8	2239	"	"	"	23·0	28·0	19·0
" 8	2239	"	"	"	22·6	29·0	17·0
" 8	2239	"	"	"	21·2	27·0	15·0
" 9	2239	"	"	"	20·6	26·0	15·0
					21·7	32·0	15·0

Table XI.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain VI., Manakumpara, from Rat 2239.

	In microns.																	
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.
Totals.	3	5	22	40	46	74	82	62	40	37	23	16	23	18	5	3	—	1
Percentages.	0·6	1·0	4·4	8·0	9·2	14·8	16·4	12·4	8·0	7·4	4·6	3·2	4·6	3·6	1·0	0·6	—	0·2

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain VI., Manakumpara, taken on 9 consecutive days from Rat 2239.

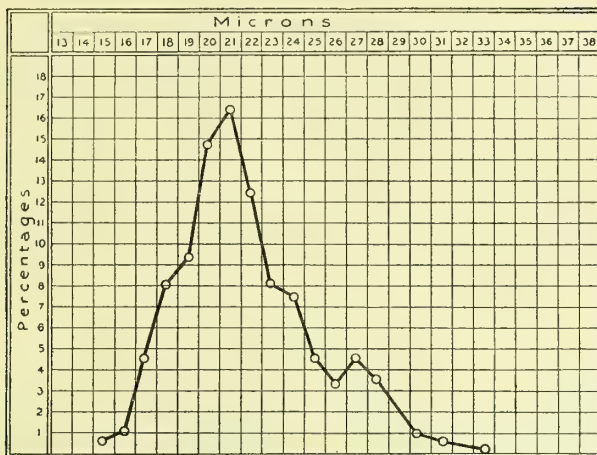


Table XII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain VI., Manakumpara.

Date.					Expt. No.	Animal.	Percentage among short and stumpy forms.
1913.							
June	30	...	...	...	2239	Rat	4
July	1	...	...	...	"	"	16
"	2	...	...	...	"	"	16
"	3	...	...	...	"	"	14
"	4	...	...	...	"	"	9
"	5	...	...	...	"	"	21
"	7	...	...	...	"	"	8
"	8	...	...	...	"	"	13
"	9	...	...	...	"	"	17
"	10	...	...	...	"	"	25
Average					...	...	14.3

Breadth.—The following table gives the breadth of the trypanosome of Strain VI., Manakumpara.

Table XIII.—Measurements of the Breadth of the Trypanosome of Strain VI., Manakumpara.

Date.	Expt. No.	Animal.	No. of trypanosomes measured.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1913	2239	Rat	500	2.76	4.50	1.25

In regard to shape, contents of cell, size and position of nucleus and micronucleus, disposal of undulating membrane and flagellum, no difference can be made out between the Strains VI. to X. and the first five strains.

Morphology of Strain VII., Yoram.

The following table gives the average length of this trypanosome as found in the white rat, 500 trypanosomes in all, and also the longest and shortest:—

Table XIV.—Measurements of the Length of the Trypanosome of Strain VII., Yoram.

Date.	Expt. No.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
June 30	2236	Rat	Osmic acid	Giemsa	25.8	28.0	23.0
" 30	2236	"	"	"	26.7	33.0	21.0
" 30	2236	"	"	"	26.9	31.0	22.0
July 1	2236	"	"	"	27.0	32.0	18.0
" 1	2236	"	"	"	26.8	32.0	21.0
" 1	2236	"	"	"	25.8	31.0	21.0
" 2	2236	"	"	"	20.3	24.0	18.0
" 2	2236	"	"	"	21.5	26.0	18.0
" 2	2236	"	"	"	21.9	29.0	19.0
" 3	2236	"	"	"	20.5	28.0	16.0
" 3	2236	"	"	"	20.2	29.0	16.0
" 3	2236	"	"	"	19.4	26.0	16.0
" 4	2236	"	"	"	19.4	23.0	17.0
" 4	2236	"	"	"	19.9	24.0	18.0
" 4	2236	"	"	"	19.5	22.0	18.0
" 5	2236	"	"	"	22.0	27.0	18.0
" 5	2236	"	"	"	21.7	30.0	17.0
" 5	2236	"	"	"	21.9	30.0	18.0
" 6	2236	"	"	"	21.7	28.0	17.0
" 6	2236	"	"	"	21.5	28.0	17.0
" 6	2236	"	"	"	22.3	28.0	18.0
" 7	2236	"	"	"	22.5	28.0	18.0
" 7	2236	"	"	"	22.5	28.0	18.0
" 7	2236	"	"	"	22.0	29.0	18.0
" 8	2236	"	"	"	22.4	34.0	18.0
					22.5	34.0	16.0

Table XV.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain VII., Yoram, from Rat 2236.

		In microns.																			
		16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	
Totals	...	3	9	40	84	67	38	47	27	28	31	33	37	21	13	13	5	2	1	1	
Percentages		0.6	1.8	8.0	16.8	13.4	7.6	9.4	5.4	5.6	6.2	6.6	7.2	4.2	2.6	2.6	1.0	0.4	0.2	0.2	

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain VII., Yoram, taken on 9 consecutive days from Rat 2236.

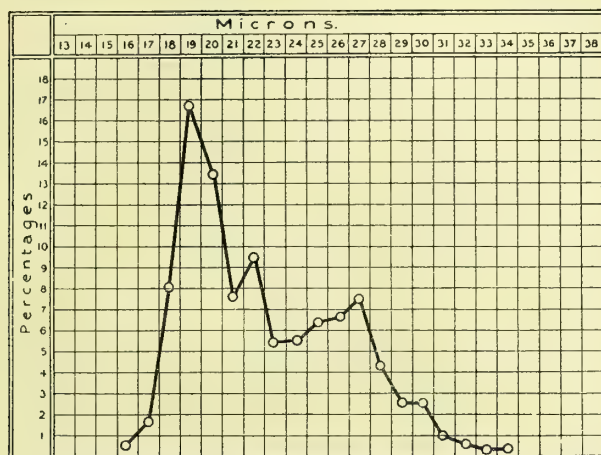


Table XVI.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain VII., Yoram.

Date.	Expt. No.	Animal.	Percentage among short and stumpy forms.
1913.			
June 30 ...	2236	Rat	0
July 1 ...	"	"	1
" 2 ...	"	"	15
" 3 ...	"	"	0
" 4 ...	"	"	2
" 5 ...	"	"	0
" 7 ...	"	"	10
" 8 ...	"	"	36
" 9 ...	"	"	34
" 10 ...	"	"	36
Average ...			13.4

Breadth.—The following table gives the breadth of the trypanosome of Strain VII., Yoramu.

Table XVII.—Measurements of the Breadth of the Trypanosome of Strain VII., Yoramu.

Date.	Expt. No.	Animal.	No. of trypanosomes measured.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1913	2236	Rat	500	2.51	4.50	1.25

Morphology of Strain VIII., Mekka.

The following table gives the average length of this trypanosome as found in the white rat, 500 trypanosomes in all, and also the longest and shortest:—

Table XVIII.—Measurements of the Length of the Trypanosome of Strain VIII., Mekka.

Date.	Expt. No.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 27	2300	Rat	Osmic acid	Giemsa	21.2	26.0	19.0
" 27	2300	"	"	"	23.2	29.0	20.0
" 27	2300	"	"	"	21.1	28.0	18.0
" 28	2300	"	"	"	22.2	27.0	19.0
" 28	2300	"	"	"	22.4	26.0	18.0
" 28	2300	"	"	"	23.4	29.0	20.0
" 29	2300	"	"	"	21.4	26.0	20.0
" 29	2300	"	"	"	23.0	28.0	21.0
" 29	2300	"	"	"	21.0	24.0	18.0
" 30	2300	"	"	"	20.4	23.0	18.0
" 30	2300	"	"	"	22.1	28.0	18.0
" 30	2300	"	"	"	20.7	25.0	18.0
" 31	2300	"	"	"	20.7	24.0	18.0
" 31	2300	"	"	"	21.4	29.0	18.0
" 31	2300	"	"	"	21.1	27.0	18.0
Sept. 1	2300	"	"	"	24.8	32.0	30.0
" 1	2300	"	"	"	23.8	33.0	20.0
" 1	2300	"	"	"	24.1	28.0	19.0
" 2	2300	"	"	"	23.9	30.0	19.0
" 2	2300	"	"	"	23.6	29.0	16.0
" 2	2300	"	"	"	23.2	30.0	20.0
" 3	2300	"	"	"	24.0	32.0	20.0
" 3	2300	"	"	"	23.1	29.0	20.0
" 3	2300	"	"	"	23.5	30.0	21.0
" 4	2300	"	"	"	21.3	27.0	18.0
					22.4	33.0	16.0

Breadth.—The following table gives the breadth of the trypanosome of Strain VIII., Mekka.

Table XXI.—Measurements of the Breadth of the Trypanosome of Strain VIII., Mekka.

Date.	Expt. No.	Animal.	No. of trypanosomes measured.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1913	2300	Rat	500	2.68	5.00	1.25

Morphology of Strain IX., Mkanthama.

The following table gives the average length of this trypanosome as found in the white rat, 500 trypanosomes in all, and also the longest and shortest:—

Table XXII.—Measurements of the Length of the Trypanosomes of Strain IX., Mkanthama.

Date.	Expt. No.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 18	2386	Rat	Osmic acid	Giemsa	23.2	31.0	17.0
" 18	2386	"	"	"	26.7	33.0	18.0
" 18	2386	"	"	"	25.1	32.0	18.0
" 19	2386	"	"	"	19.0	24.0	17.0
" 19	2386	"	"	"	18.5	26.0	16.0
" 19	2386	"	"	"	18.2	23.0	16.0
" 20	2386	"	"	"	19.4	24.0	17.0
" 20	2386	"	"	"	19.8	30.0	16.0
" 20	2386	"	"	"	19.9	30.0	16.0
" 21	2386	"	"	"	17.7	24.0	15.0
" 21	2389	"	"	"	17.8	22.0	14.0
" 21	2386	"	"	"	17.5	20.0	16.0
" 22	2386	"	"	"	19.4	31.0	15.0
" 22	2386	"	"	"	20.5	28.0	15.0
" 22	2386	"	"	"	21.8	29.0	15.0
" 23	2386	"	"	"	23.5	29.0	17.0
" 23	2386	"	"	"	24.2	32.0	16.0
" 23	2386	"	"	"	23.4	31.0	16.0
" 24	2386	"	"	"	24.1	33.0	18.0
" 24	2386	"	"	"	23.4	31.0	16.0
" 24	2386	"	"	"	21.4	30.0	17.0
" 25	2386	"	"	"	22.0	29.0	17.0
" 25	2386	"	"	"	21.8	31.0	17.0
" 25	2386	"	"	"	21.2	30.0	15.0
" 26	2386	"	"	"	19.6	26.0	17.0
					21.2	33.0	14.0

Table XXIII.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain IX., Mkanthama.

	In microns.																						
	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.			
Totals...	1	9	22	64	79	7	40	23	21	29	23	24	23	16	13	15	12	9	5	2			
Per- centages	0.2	1.0	4.4	12.8	15.8	1.4	8.0	4.6	4.2	5.8	4.6	4.8	4.6	3.2	2.6	3.0	2.4	1.6	1.0	0.4			

CHART 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain IX., Mkanthama, taken on 9 consecutive days from Rat 2386.

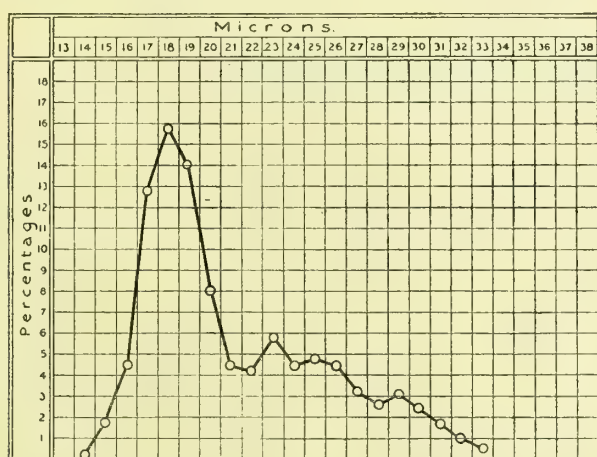


Table XXIV.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IX., Mkanthama.

Date.		Expt. No.	Animal.	Percentage among short and stumpy forms.
1913.				
May 18 ...	...	2386	Rat	13
" 19 ...	...	"	"	32
" 20 ...	...	"	"	18
" 21 ...	...	"	"	11
" 22 ...	...	"	"	19
" 23 ...	...	"	"	27
" 24 ...	...	"	"	34
" 25 ...	...	"	"	33
" 26 ...	...	"	"	45
" 28 ...	...	"	"	54
Average ...				28.6

Breadth.—The following table gives the breadth of the trypanosome of Strain IX., Mkanthama.

Table XXV.—Measurements of the Breadth of the Trypanosome of Strain IX., Mkanthama.

Date.	Expt. No.	Animal.	No. of trypanosomes measured.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1913	2386	Rat	500	2.56	5.00	1.25

Morphology of Strain X., Dongolosi.

The following table gives the average length of this trypanosome, as found in the white rat, 500 trypanosomes in all, and also the longest and shortest:—

Table XXVI.—Measurements of the Length of the Trypanosome of Strain X., Dongolosi.

Date.	Expt. No.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Nov. 27	2437	Rat	Osmic acid	Giemsa	24.8	30.0	20.0
" 27	2437	"	"	"	24.6	29.0	21.0
" 27	2437	"	"	"	25.0	29.0	21.0
" 28	2437	"	"	"	19.9	22.0	17.0
" 28	2437	"	"	"	19.9	23.0	18.0
" 28	2437	"	"	"	19.6	22.0	18.0
Dec. 2	2437	"	"	"	23.2	28.0	17.0
" 2	2437	"	"	"	22.0	29.0	18.0
" 2	2437	"	"	"	23.2	28.0	18.0
" 3	2437	"	"	"	24.0	30.0	20.0
" 3	2437	"	"	"	23.7	29.0	20.0
" 3	2437	"	"	"	24.0	30.0	19.0
" 4	2437	"	"	"	23.2	27.0	19.0
" 4	2437	"	"	"	23.2	30.0	19.0
" 4	2437	"	"	"	23.7	26.0	21.0
" 5	2437	"	"	"	24.3	28.0	20.0
" 5	2437	"	"	"	23.9	29.0	19.0
" 5	2437	"	"	"	24.4	28.0	21.0
" 6	2437	"	"	"	24.9	30.0	20.0
" 6	2437	"	"	"	25.2	30.0	22.0
" 6	2437	"	"	"	24.5	28.0	21.0
" 7	2437	"	"	"	25.3	31.0	20.0
" 7	2437	"	"	"	25.0	30.0	20.0
" 7	2437	"	"	"	24.8	32.0	20.0
" 8	2437	"	"	"	24.3	25.0	18.0
					23.5	32.0	17.0

Table XXVII.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain X., Dongolosi.

	In microns.															
	17.	18.	19.	20	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.
Totals...	2	9	30	36	51	52	69	68	61	48	25	23	11	12	2	1
Per- centages	0.4	1.8	6.0	7.2	10.2	10.4	13.8	13.6	12.2	9.6	5.0	4.6	2.2	2.4	0.4	0.2

CHART 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain X., Dongolosi, taken on 9 consecutive days from Rat 2437, at the beginning of the infection.

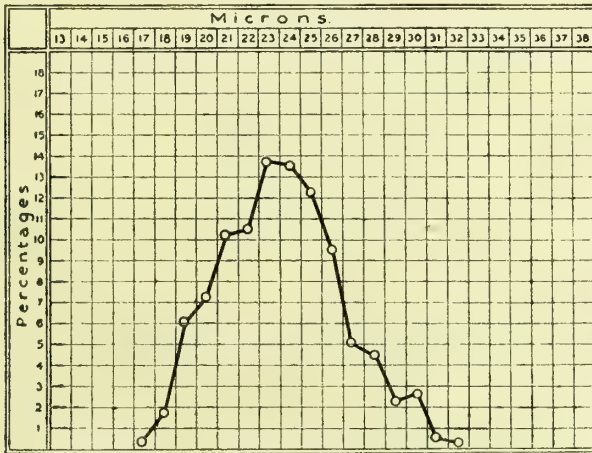
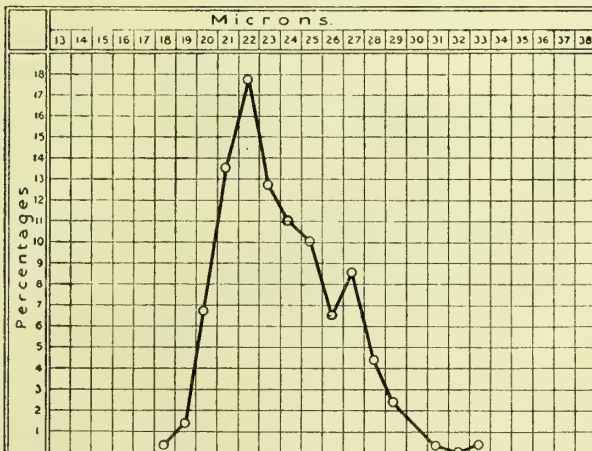


CHART 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain X., Dongolosi, taken on the 33rd and 41st day of disease from Rat 2462.



It is evident that in this case there is little difference in type in the trypanosomes taken during the first 9 days and the last 9 days of an infection. In the former there are 25 per cent. short and stumpy forms, 37 per cent. intermediate, and 38 per cent. long and slender; in the latter 22, 43, and 35 per cent. respectively.

Table XXVIII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of Trypanosome of Strain X., Dongolosi.

Date.				Expt. No.	Animal.	Percentage among short and stumpy forms.
1913.						
November	27	...	...	2437	Rat	1
"	28	...	...	"	"	3
December	2	...	...	"	"	0
"	3	...	...	"	"	1
"	4	...	...	"	"	10
"	5	...	...	"	"	3
"	6	...	...	"	"	4
"	7	...	...	"	"	7
"	8	...	...	"	"	10
"	9	...	...	"	"	11
Average				...	...	5.0

Breadth.—The following table gives the breadth of Strain X. Dongolosi.

Table XXIX.—Measurements of the Breadth of the Trypanosome of Strain X., Dongolosi.

Date.	Expt. No.	Animal.	No. of trypanosomes measured.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1913	2437	Rat	500	2.71	4.50	1.25

To Ascertain the Type of Trypanosomes which arises from a single Trypanosome.

There is always, when dealing with a dimorphic type of trypanosome, a danger of there being two species present. Some experiments were therefore made by inoculating animals with a single trypanosome, to find out if the original dimorphic type would appear. The single trypanosomes were picked out in the usual way, by means of dilution and capillary tubes. The blood of an infected rat was diluted with normal saline solution until a volume of one-sixteenth of an inch in length of a fine capillary tube was found to contain a single trypanosome. This was then inoculated into a rat. Six experiments were made, three of which were successful. In each case the long and slender type of trypanosome was isolated and injected.

As will be seen from the following curves, from the single long and slender type, short and stumpy, intermediate, and long and slender forms resulted. Among the short and stumpy there was a large percentage of the blunt-ended posterior-nucleated forms, which are a feature of this species of trypanosome.

From these three experiments, then, it may be concluded that in the dimorphic Trypanosome causing Disease in Man in Nyasaland, a single species is being dealt with.

CHART 7.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain X., Dongolosi, taken on 9 consecutive days from Rat 2493, which had been infected by a single trypanosome of the long and slender type.

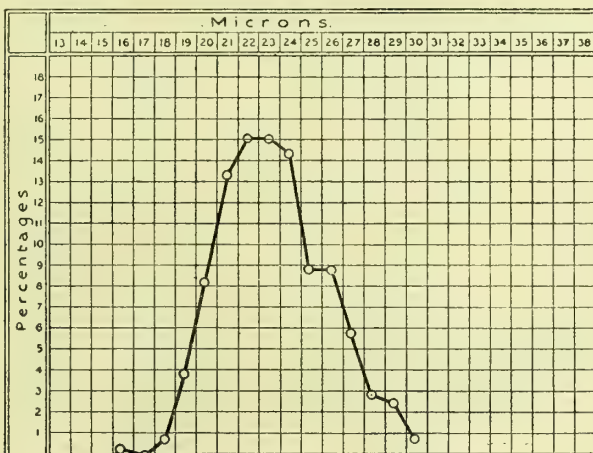
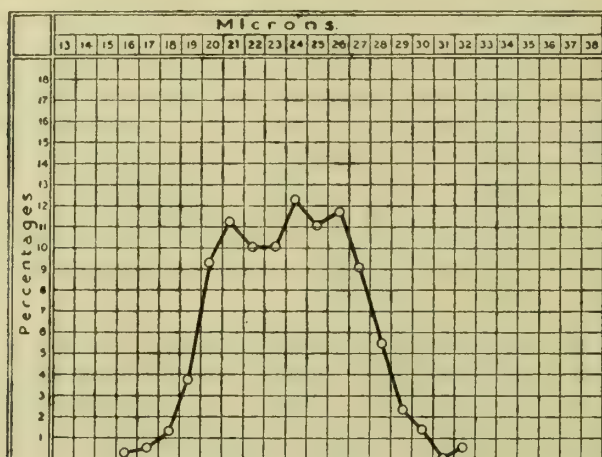


CHART 8.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain X., Dongolosi, taken on 9 consecutive days from Rat 2489, which had been infected by a single trypanosome of the long and slender type.



Comparison of the Human Strains VI. to X.

Table XXX.—Measurements of the Length of the Trypanosomes of the Five Human Strains VI. to X. The trypanosomes have been taken from the rats alone.

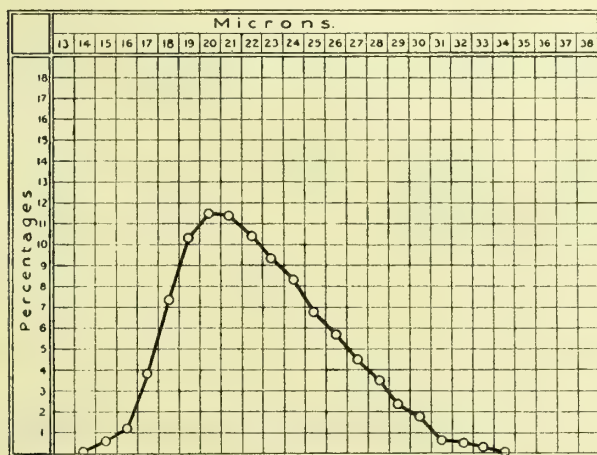
Date.	Strain.	Name.	No. of trypanosomes measured.	In microns.		
				Average length.	Maximum length.	Minimum length.
1913.	VI.	Manakumpara	500	21.7	32.0	15.0
"	VII.	Yoramu	500	22.5	34.0	16.0
"	VIII.	Mekka	500	22.4	33.0	16.0
"	IX.	Mkanthama	500	21.2	33.0	14.0
"	X.	Dongolosi	500	23.5	32.0	17.0
				22.3	34.0	14.0

The average length of Strains I. to V., taken from rats alone, is 24.2 microns; maximum 38.0, minimum 15.0. This gives an average for the 10 strains of 23.2 microns; maximum 38.0, minimum 14.0.

Table XXXI.—Distribution in respect to Length of 2,500 Individuals of the Human Strains VI. to X. The trypanosomes have been taken from the rat alone.

	In microns.																											
	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.							
Totals ..	1	12	31	97	187	260	286	283	262	233	205	173	140	109	85	57	45	18	11	4	1							
Per- centages	0.1	0.5	1.2	3.9	7.5	10.4	11.4	11.3	10.5	9.3	8.2	6.9	5.6	4.3	3.4	2.3	1.8	0.7	0.4	0.2	0.1							

CHART 9.—Curve representing the Distribution, by Percentages, in respect to Length, of 2,500 Individuals of the Human Strains VI. to X. of the Trypanosome Causing Disease in Man in Nyasaland, taken from the rat alone.



This curve is very similar to that made from Strains I. to V.,* except that it lies a little to the shorter side.

* "Proc." Roy. Soc., B, vol. 86, 1913, p. 301, and Reports of the Sleeping Sickness Commission, No. XV., p. 47.

Table XXXII.—Comparison of the Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Human Strains VI. to X.

Date.	Expt. No.	Strain.	Animal.	Percentage among short and stumpy forms.
1913	2239	VI. Manakumpara ...	Rat	14·3
1913	2236	VII. Yoramu ...	"	13·4
1913	2300	VIII. Mekka ...	"	24·2
1913	2386	IX. Mkanthama ...	"	28·6
1913	2437	X. Dongolosi ...	"	5·0
Average ...				17·1

The average percentage of the Strains I. to V. was 17·8 microns.

Table XXXIII.—Comparison of the Measurements of the Breadth of the Trypanosomes of the Human Strains VI. to X.

Date.	Expt. No.	Strain.	Animal.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1913	2239	VI. Manakumpara	Rat	2·76	4·50	1·25
1913	2236	VII. Yoramu ...	"	2·51	4·50	1·25
1913	2300	VIII. Mekka ...	"	2·68	5·00	1·25
1913	2386	IX. Mkanthama ...	"	2·56	5·00	1·25
1913	2437	X. Dongolosi ...	"	2·71	4·50	1·25
				2·65	5·00	1·25

Conclusion.

These further five strains of the trypanosomes, isolated from five natives in Nyasaland, belong to the same species, *Trypanosoma brucei vel rhodesiense*, the Trypanosome causing Disease in Man in Nyasaland.

XVI.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME—*TRYPANOSOMA BRUCEI VEL RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE WILD GAME STRAIN. STRAINS XI. to XV.

This paper has already been published in the "Proceedings" of the Royal Society, B, vol. 86, 1913, p. 394, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 48.

Trypanosomes of this species, isolated from 5 antelope shot in the Sleeping Sickness Area, are described in the paper. They are compared with strains obtained from natives suffering from Nyasaland sleeping sickness, and the conclusion is come to that the trypanosomes found in the blood of the wild animals are identical with those found in the blood of man, and that this species is *Trypanosoma brucei* vel *rhodesiense*.

XVII.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE WILD *GLOSSINA MORSITANS* STRAIN. STRAINS XVI. to XX.

This paper has previously been published in the "Proceedings" of the Royal Society, B, vol. 86, 1913, p. 408, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 61.

Five strains were obtained from the tsetse fly (*Glossina morsitans*) caught in the neighbouring fly country. These are compared with strains from man and the wild game, and it is concluded that the trypanosomes from all three sources belong to the same species of trypanosome—*Trypanosoma brucei* vel *rhodesiense*.

XVIII.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE MZIMBA STRAIN. STRAIN XXI.

This paper also has been published in the "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 26, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 103.

This is another strain of the same trypanosome—*Trypanosoma brucei* vel *rhodesiense*—but obtained in this case from Mzimba, about 100 miles north of Kasu camp. It probably came from the tsetse fly of that district, as it was found in the blood of an infected donkey which had been exposed to the bite of the fly.

XIX.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE LIWONDE STRAIN. STRAINS XXII. TO XXIV.*

This strain was obtained from tsetse flies in the fly-area of the Upper Shire valley, near Liwonde, in the Upper Shire district, about 100 miles south of the Proclaimed or Sleeping Sickness Area.† At

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 97.

† "Trypanosomes Found in Wild *Glossina morsitans* and Wild Game in the Fly-Belt of the Upper Shire Valley," "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 38. (See also pp. 10 and 21 of this Report.)

the time it was procured no cases of trypanosome disease in man had been reported from this district; lately, a case has been discovered near Mpimbi (lat. $15^{\circ} 12'$ S., long. $34^{\circ} 10'$ E.), in the south of the district, about 150 miles south of the Proclaimed or Sleeping Sickness Area, and within 20 miles of Zomba, the official cantonment.

Three dogs infected with the wild *Glossina morsitans* strain of this trypanosome were brought to Kasu, and other animals—monkeys, dogs, and rats—were inoculated from them.

For purposes of description, measurements and comparison, only trypanosomes from rats were used.

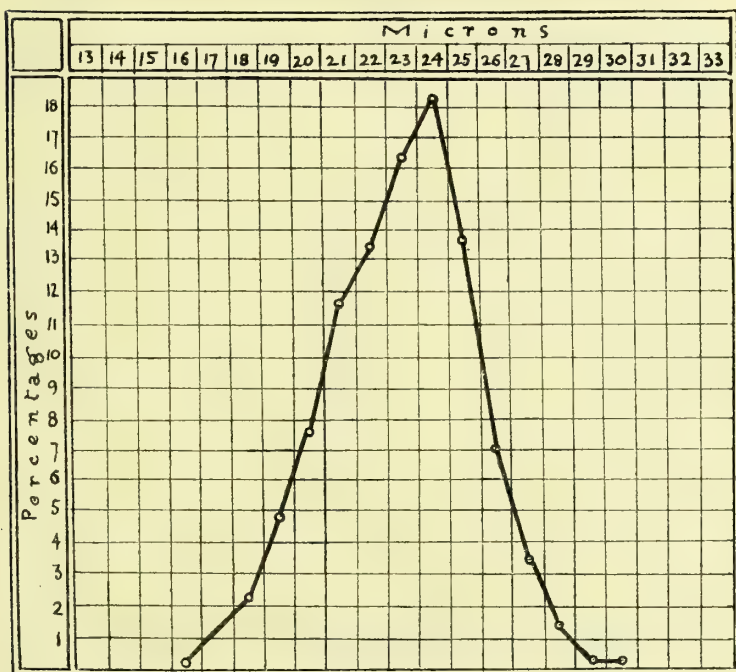
Morphology of the First Liwonde Strain. Strain XXII.

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all.

Table XXXIV.—Measurements of the Length of the Trypanosome of the Liwonde Strain XXII.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 18	2378	Rat	Osmic acid	Giemsa	24.8	28.0	19.0
" 18	2378	"	"	"	24.3	27.0	21.0
" 18	2378	"	"	"	24.6	27.0	22.0
" 19	2378	"	"	"	23.4	27.0	20.0
" 19	2378	"	"	"	23.4	27.0	20.0
" 19	2378	"	"	"	24.7	27.0	21.0
" 20	2378	"	"	"	22.3	25.0	19.0
" 20	2378	"	"	"	21.4	25.0	18.0
" 20	2378	"	"	"	21.3	24.0	18.0
" 21	2378	"	"	"	22.1	26.0	16.0
" 21	2378	"	"	"	22.1	26.0	19.0
" 21	2378	"	"	"	21.2	25.0	18.0
" 22	2378	"	"	"	23.3	28.0	20.0
" 22	2378	"	"	"	23.1	26.0	20.0
" 22	2378	"	"	"	23.2	28.0	20.0
" 23	2378	"	"	"	23.3	28.0	21.0
" 23	2378	"	"	"	24.0	28.0	20.0
" 23	2378	"	"	"	24.2	30.0	20.0
" 24	2378	"	"	"	23.0	28.0	20.0
" 24	2378	"	"	"	23.1	29.0	19.0
" 24	2378	"	"	"	22.9	25.0	19.0
" 25	2378	"	"	"	22.5	27.0	18.0
" 25	2378	"	"	"	22.0	26.0	18.0
" 25	2378	"	"	"	22.0	25.0	18.0
" 26	2378	"	"	"	21.8	24.0	18.0
					23.0	30.0	16.0

CHART 10.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain XXII., taken on 9 consecutive days from Rat 2378.



This curve differs from the ordinary wild *Glossina morsitans* curve, but is similar to Strains II., IV., and V. of the Human Strain.*

Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all.

Table XXXV.—Measurements of the Breadth of the Trypanosome of the Liwonde Strain XXII., measured across the widest part, including the undulating membrane.

Expt. No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
2378	Rat	500	3.0	4.50	1.25

* "Proc." Roy. Soc., B, vol. 86, 1913, pp. 288, 295, and 298.

Table XXXVI.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain XXII.

Date.					Expt. No.	Animal.	Percentage among short and stumpy forms.
1913.							
Aug. 18	...	...	...	...	2378	Rat	2
" 19	...	...	...	...	2378	"	23
" 20	...	...	...	...	2378	"	17
" 21	...	...	...	...	2378	"	4
" 22	...	...	...	...	2378	"	12
" 23	...	...	...	...	2378	"	16
" 24	...	...	...	...	2378	"	28
" 25	...	...	...	...	2378	"	15
" 26	...	...	...	...	2378	"	19
" 27	...	...	...	...	2378	"	22
Average					...	...	15.8

Morphology of the Second Liwonde Strain. Strain XXIII.

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all.

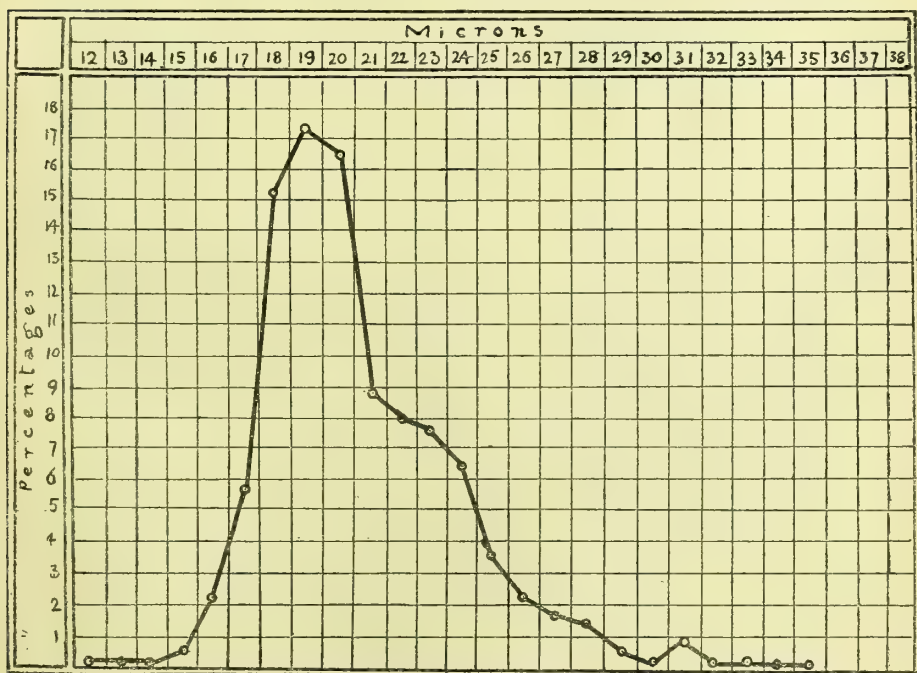
Table XXXVII.—Measurements of the Length of the Trypanosome of the Liwonde Strain XXIII.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 18	2363	Rat	Osmic acid	Giemsa	20.3	31.0	17.0
" 18	2363	"	"	"	19.0	23.0	14.0
" 18	2363	"	"	"	20.3	26.0	18.0
" 19	2363	"	"	"	19.2	25.0	18.0
" 19	2363	"	"	"	20.6	25.0	17.0
" 19	2363	"	"	"	19.7	24.0	17.0
" 20	2363	"	"	"	20.7	25.0	18.0
" 20	2363	"	"	"	21.2	29.0	18.0
" 20	2363	"	"	"	20.0	24.0	17.0
" 21	2363	"	"	"	19.0	24.0	16.0
" 21	2363	"	"	"	19.9	28.0	13.0
" 21	2363	"	"	"	19.0	26.0	17.0

Table XXXVII. (continued).

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 22	2363	Rat	Osmic acid	Giemsa	21.9	27.0	18.0
" 22	2363	"	"	"	21.9	28.0	18.0
" 22	2363	"	"	"	22.9	27.0	20.0
" 23	2363	"	"	"	22.6	33.0	18.0
" 23	2363	"	"	"	22.9	34.0	18.0
" 23	2363	"	"	"	21.0	30.0	17.0
" 24	2363	"	"	"	20.9	35.0	17.0
" 24	2363	"	"	"	22.2	31.0	16.0
" 24	2363	"	"	"	20.1	31.0	16.0
" 25	2363	"	"	"	20.9	28.0	16.0
" 25	2363	"	"	"	20.5	28.0	15.0
" 25	2363	"	"	"	20.6	27.0	12.0
" 26	2363	"	"	"	20.1	31.0	17.0
					20.7	35.0	12.0

CHART 11.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain XXIII., taken on 9 consecutive days from Rat 2363.



Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all.

Table XXXVIII.—Measurements of the Breadth of the Trypanosome of the Liwonde Strain XXIII. ; measured across the widest part, including the undulating membrane.

Expt. No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
2363	Rat	500	3.0	4.75	1.25

Table XXXIX.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain XXIII.

Date.					Expt. No.	Animal.	Percentage among short and stumpy forms.
1913.							
August 18	...	...	...	...	2363	Rat	25
" 19	...	...	...	...	2363	"	13
" 20	...	...	...	...	2363	"	14
" 21	...	...	...	...	2363	"	16
" 22	...	...	...	...	2363	"	12
" 23	...	...	...	...	2363	"	23
" 24	...	...	...	...	2363	"	35
" 25	...	...	...	...	2363	"	37
" 26	...	...	...	...	2363	"	14
" 27	...	...	...	...	2363	"	23
Average					...	...	21.2

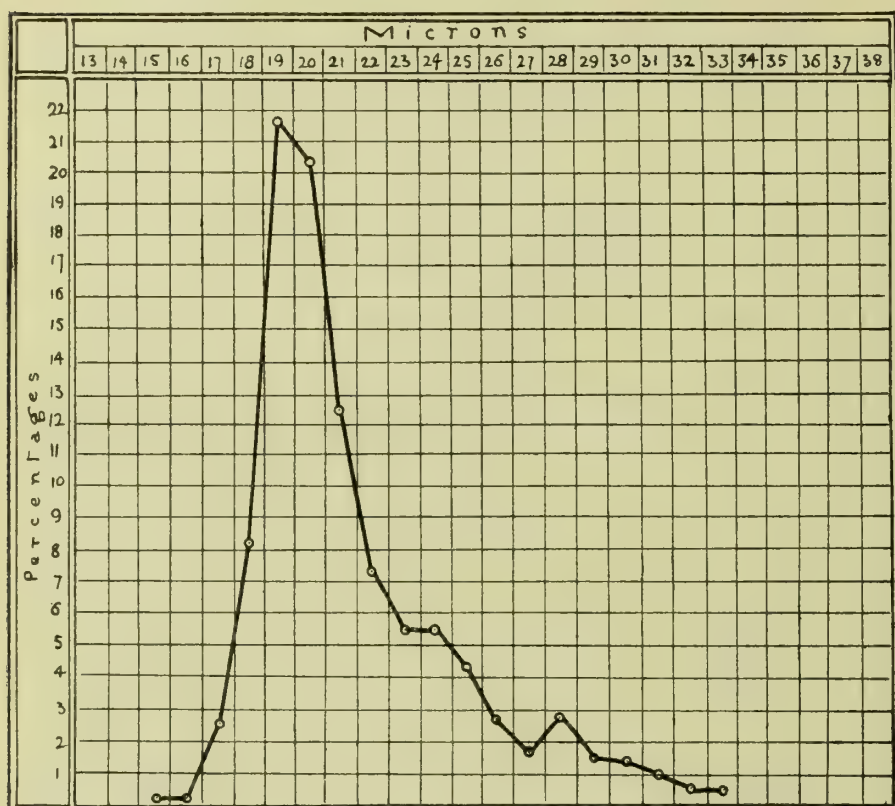
Morphology of the Third Liwonde Strain. Strain XXIV.

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all.

Table XL.—Measurements of the Length of the Trypanosome of the Liwonde Strain XXIV.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 18	2370	Rat	Osmic acid	Giemsa.	22·9	29·0	18·0
" 18	2370	"	"	"	22·1	30·0	17·0
" 18	2370	"	"	"	22·6	31·0	18·0
" 19	2370	"	"	"	19·7	24·0	18·0
" 19	2370	"	"	"	19·8	23·0	18·0
" 19	2370	"	"	"	19·9	28·0	18·0
" 20	2370	"	"	"	20·9	25·0	19·0
" 20	2370	"	"	"	21·2	30·0	18·0
" 20	2370	"	"	"	22·6	32·0	19·0
" 21	2370	"	"	"	20·0	24·0	17·0
" 21	2370	"	"	"	21·0	28·0	17·0
" 21	2370	"	"	"	20·3	28·0	17·0
" 22	2370	"	"	"	19·9	29·0	17·0
" 22	2370	"	"	"	20·1	25·5	15·0
" 22	2370	"	"	"	20·5	29·0	17·0
" 23	2370	"	"	"	23·7	30·0	19·0
" 23	2370	"	"	"	23·9	32·0	18·0
" 23	2370	"	"	"	23·0	30·0	17·0
" 24	2370	"	"	"	21·7	33·0	17·0
" 24	2370	"	"	"	23·0	33·0	18·0
" 24	2370	"	"	"	22·2	27·0	18·0
" 25	2370	"	"	"	21·7	31·0	18·0
" 25	2370	"	"	"	21·0	30·0	18·0
" 25	2370	"	"	"	20·9	29·0	17·0
" 26	2370	"	"	"	19·7	26·0	16·0
					21·4	33·0	15·0

CHART 12.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain XXIV., taken on nine consecutive days from Rat 2370.



Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all.

Table XLI.—Measurements of the Breadth of the Trypanosome of the Liwonde Strain XXIV., measured across the widest part, including the undulating membrane.

Expt. No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
2370	Rat	500	2.6	4.75	1.25

Table XLII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain XXIV.

Date.					Expt. No.	Animal.	Percentage among short and stumpy forms.
1913.							
August 18	...	...	...	...	2370	Rat	4
" 19	...	...	...	...	2370	"	44
" 20	...	...	...	...	2370	"	36
" 21	...	...	...	...	2370	"	33
" 22	...	...	...	...	2370	"	22
" 23	...	...	...	...	2370	"	4
" 24	...	...	...	...	2370	"	6
" 25	...	...	...	...	2370	"	12
" 26	...	...	...	...	2370	"	30
" 27	...	...	...	...	2370	"	33
Average ...					...	...	22.4

Comparison of the Three Liwonde Strains with one another.

Table XLIII.—Measurements of the Length of the Trypanosome of the Liwonde Strains, XXII. to XXIV.

Date.	Expt. No.	Strain.	Animal.	No. of trypanosomes measured.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913	2378	XXII.	Rat	500	23.0	30.0	16.0
1913	2363	XXIII.	"	500	20.7	35.0	12.0
1913	2370	XXIV.	"	500	21.4	33.0	15.0
					21.7	35.0	12.0

CHART 13.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,500 Individuals of the Trypanosome of the Liwonde Strains XXII. to XXIV.

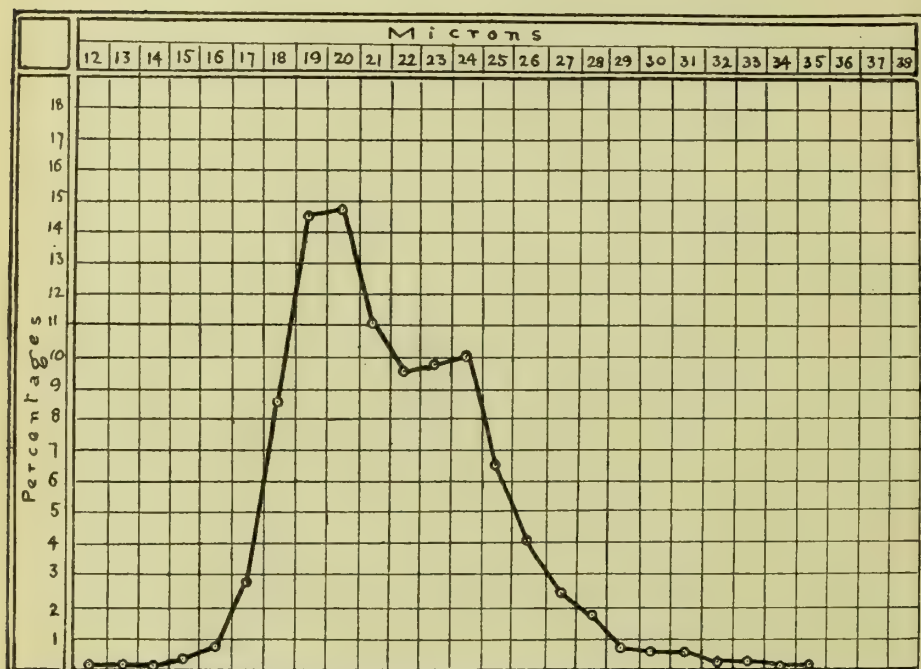


Table XLIV.—Measurements of the Breadth of the Trypanosome of the three Liwonde Strains.

Date.	Expt. No.	Strain.	Animal.	No. of trypanosomes measured.	In microns.		
					Average breadth.	Maximum breadth.	Minimum breadth.
1913	2378	XXII	Rat	500	3.0	4.50	1.25
1913	2363	XXIII	"	500	3.0	4.75	1.25
1913	2370	XXIV	"	500	2.6	4.75	1.25
					2.9	4.75	1.25

Table XLV.—Percentages of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the three Liwonde Strains.

Date.	Expt. No.	Strain.	Animal.	Percentage among short and stumpy forms.
1913	2378	XXII.	Rat	15·8
1913	2363	XXIII.	„	21·2
1913	2370	XXIV.	„	22·4
Average ...				19·6

Comparison of the Liwonde Strain with the Human, Wild Game and Wild Glossina morsitans Strains of the Trypanosome causing Disease in Man in Nyasaland.

Table XLVI.—Average length of the Trypanosome of the Human, Wild Game, Wild *Glossina morsitans* and Liwonde Strains.

Strain.	No. of trypanosomes measured.	Animal.	In microns.		
			Average length.	Maximum length.	Minimum length.
Human... ..	5500	Rat	23·5	38·0	14·0
Wild game ...	2500	„	22·6	35·0	15·0
Wild <i>G. morsitans</i>	2500	„	22·6	35·0	15·0
Liwonde ...	1500	„	21·7	35·0	12·0

CHART 14.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,500 Individuals of the Trypanosome of the Liwonde Strain, 5,000 of the Wild Game and Wild *Glossina morsitans* Strains combined, and 5,500 of the Human Strain, all measured from rats.

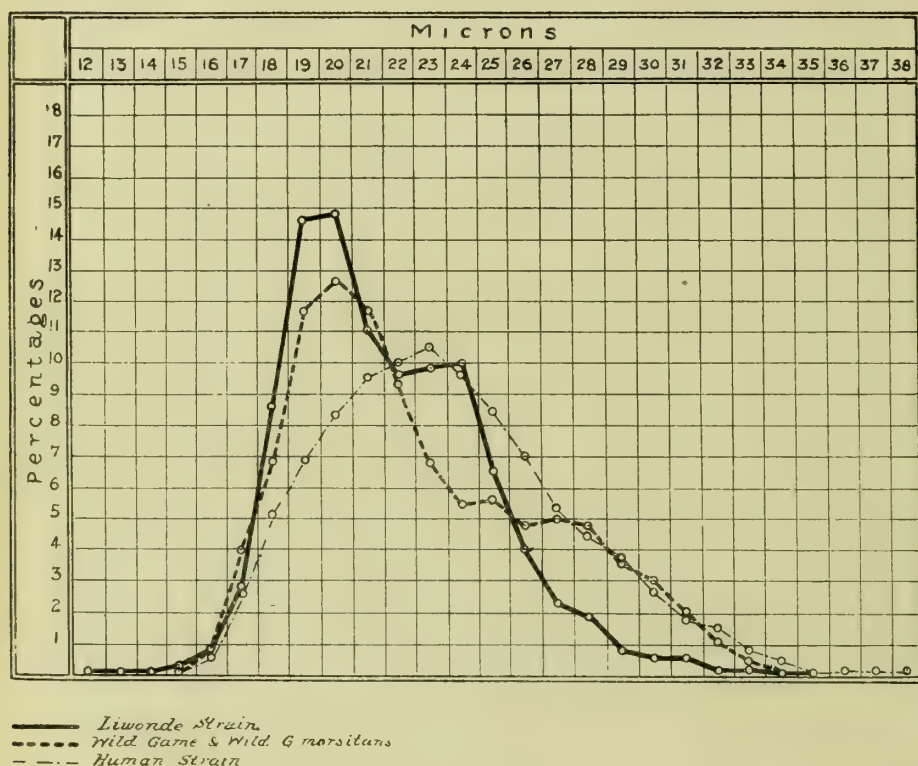


Table XLVII.—Average Breadth of the Trypanosome of the Human, Wild Game, Wild *Glossina morsitans*, and Liwonde Strains.

Strain.	No. of trypanosomes measured.	Animal.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
Human ...	1500	Rat	2.6	5.0	1.25
Wild game ...	1500	"	3.2	5.75	1.50
Wild <i>G. morsitans</i> ...	1500	"	2.9	5.25	1.25
Liwonde ...	1500	"	2.9	4.75	1.25
			2.9	5.75	1.25

Table XLVIII.—Percentages of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human, Wild Game, Wild *Glossina morsitans* and Liwonde Strains.

Strain.	Animal.	Percentage among short and stumpy forms.
Human	Rat	17·8
Wild Game	"	26·2
Wild <i>G. morsitans</i>	"	12·5
Liwonde... ..	"	19·8
Average		19·1

Conclusions.

1. The three wild *Glossina morsitans* strains from the neighbourhood of Liwonde resemble each other closely, and all belong to the same species of trypanosome.

2. The Liwonde strain belongs to the same species as that occurring in man, wild game, and wild *Glossina morsitans* inhabiting the Proclaimed Area, Nyasaland—*Trypanosoma brucei vel rhodesiense*.

3. Hence it would appear that wild *Glossina morsitans* occurring in a district 100 miles south of the Proclaimed Area are infected with the trypanosome which causes the Human Trypanosome Disease of Nyasaland.

XX.—MORPHOLOGY OF VARIOUS STRAINS OF THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN. THE ZULU-LAND, 1913, STRAIN. STRAIN XXV.*

In July, 1912, Sir Arnold Theiler, K.C.M.G., Director of Veterinary Research (Union of South Africa), Pretoria, sent this Commission several blood preparations taken from horses and dogs supposed to be suffering from nagana. Much to the surprise of the Commission, a large percentage of these trypanosomes showed posterior-nuclear forms. This disposed of the contention that the

* Reprinted, with amendments, from the "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 493.

so-called *Trypanosoma rhodesiense* could be distinguished from other species of trypanosomes by this peculiarity, and first led the Commission to suspect that *T. rhodesiense* was in reality *T. brucei*.

Sir Arnold was then asked to send the living strain through to the Commission in Nyasaland, and this, after several failures, was at last successfully carried out.

The history of the strain is as follows: Mr. A. W. Shilston, Veterinary Research Division, Pietermaritzburg, writes that it originated in a mule which was naturally infected at Somkele in Zululand. A dog was inoculated from this mule and brought to the Veterinary Research Laboratory at Pietermaritzburg, where sub-inoculations into a series of animals were made. Mr. Shilston says there is no possibility of this strain having been mixed with any other, as at the time he was working at it, it was the only species of trypanosome maintained at the laboratory. He also states that he—in order to prove that he was dealing with a single species of trypanosome and not with a mixed infection—infected rabbits with single parasites, and the resulting infections showed the same morphological characters as the original strain.

From Pietermaritzburg the strain was transferred to Pretoria. Mr. William Robertson, acting director during the absence on leave of Sir Arnold Theiler, informs the Commission that the strain was kept going in Pretoria in horses and cattle, in which animals it produced the typical clinical symptoms and *post-mortem* lesions associated with nagana, and that it was always regarded as a pure uncomplicated strain of *T. brucei*. The thanks of the Commission are due to Mr. Robertson for his perseverance in sending inoculated animals to their camp at Kasu. Like the Japanese general outside Port Arthur, as one batch succumbed he sent on another, until at last he succeeded.

The exact length of time this trypanosome was kept going at Pretoria before being sent to Kasu is not given, but the information has been asked for, and will be placed on record as soon as obtained.

In the opinion of the Commission, the trypanosome dealt with in this paper is the same as that discovered by Bruce in Zululand in 1894, and named *T. brucei* by Plimmer and Bradford. Somkele is in the same district in Zululand as that in which this species of trypanosome was first discovered.

In this paper the old Zululand strain will be called the 1896 strain, that being the year in which it was first described; the new strain (No. XXV.), the 1913 strain, the year in which it was received from Pretoria.

The Zululand trypanosomes were described by Bruce in his original paper* as hæmatozoa which vary among themselves a good deal in size and shape. Photographs were also given, which show a distinct dimorphic type. In a later paper† Bruce gives measurements of

* "Further Report on the Tsetse-fly Disease, or Nagana, in Zululand," 1896.

† "Proc." Roy. Soc., B. vol. 83, p. 9 (1910).

200 trypanosomes taken from preparations which had been made in Zululand in 1896, and also gives six figures taken from the same source. From these it will be seen that the trypanosome dealt with in Zululand in 1896 was a markedly dimorphic form, with long and slender, intermediate, and short and stumpy forms. From the above measurements and figures there cannot be the slightest shadow of doubt about this.

In 1896 Bruce sent this trypanosome to England, and it was at once placed in the hands of Kanthack, Durham, and Blandford by the Royal Society to be reported on. Their investigation lasted two years, and was published in vol. 64 of the "Proceedings" of the Royal Society. In regard to the shape of this trypanosome they state that "the Nagana parasites vary considerably both in size and form; they may be long and pointed or blunt-ended and somewhat stouter; some individuals are short and thick, with a short flagellum, their protoplasm being crowded with rounded granules." No one who reads Bruce's "Progress Report" and compares it with Kanthack, Durham, and Blandford's 1898 report can doubt that the same trypanosome was being dealt with. This trypanosome was distinctly dimorphic.

About this time (1898) the trypanosome was handed over to Bradford at the Brown Institute and named *T. brucei* in a paper written in 1899.*

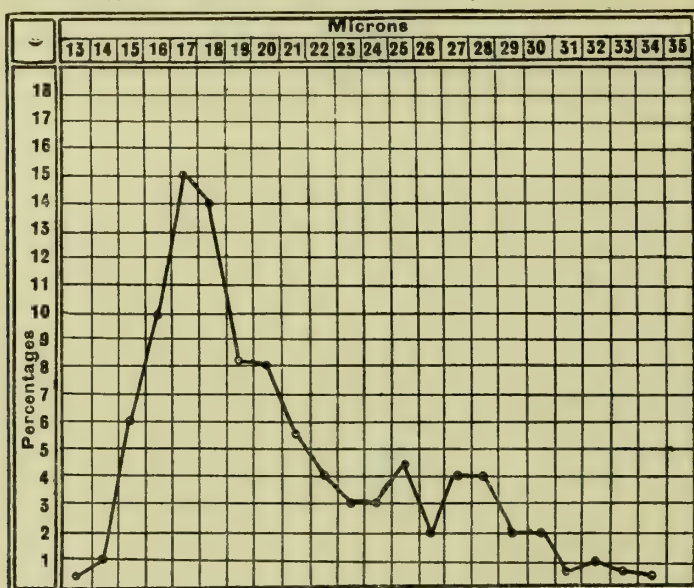
That the trypanosome named by Plimmer and Bradford was the same as that sent to Kanthack, Durham, and Blandford in 1896 there can be no reasonable doubt. There was no other species of pathogenic trypanosome in any English laboratory at the time, with the exception, perhaps, of *T. Lewisi*, with which there could be no confusion.

Now, having shown that the original Zululand strain was a well-marked dimorphic type of trypanosome, let us see how it compares with the 1913 strain (No. XXV.).

Mr. Shilston kindly sent the Commission a description of this strain made immediately after it had come from Zululand. He states that in the living condition the variation in size and shape of the organism can be observed, the long, slender flagellated forms being readily distinguished from the short, stumpy forms, while all gradations between these two can be found; that the circular vacuole close to the micronucleus is very distinct; and that, although the organisms are actively motile their progression is not rapid, and frequently they simply travel in a small circle.

* "Preliminary Note on the Morphology and Distribution of the Organism found in the Tsetse-fly Disease," by H. G. Plimmer and J. Rose Bradford, "Proc." Roy. Soc., vol. 65, p. 274.

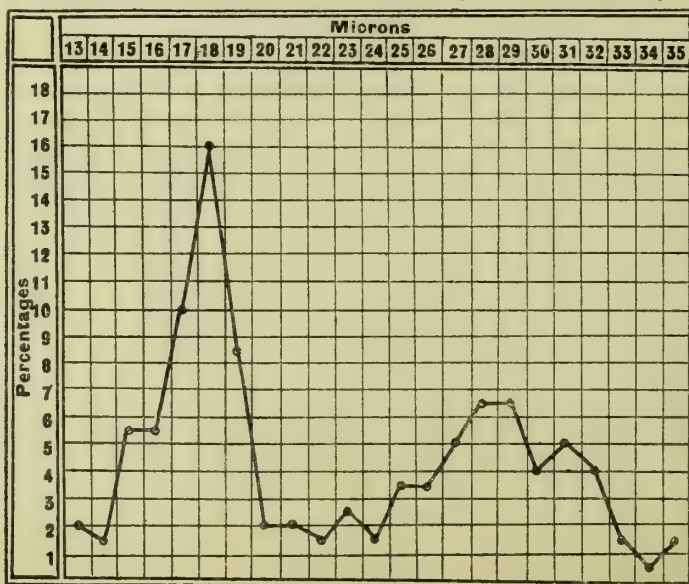
CHART 15.—Curve representing the Distribution, by Percentages, in respect to Length, of 400 Individuals of *T. brucei*, Zululand Strain (No. XXV.), 1913 (Shilston's measurements).



Mr. Shilston also made a large number of measurements of this strain. One of his charts gives the percentages in respect to length of 400 trypanosomes occurring in the mule, dog, and guinea-pig, the measurements being made at varying periods of the disease. This chart is reproduced above.

In a previous paper* a curve will be found representing the percentages in respect to length of 200 individuals of the original strain of *T. brucei*, measured from old Zululand preparations which had been made in 1896 and were still extant. The numbers making up these two curves are doubtless small, but they are fairly comparable.

CHART 16.—Curve representing the Distribution, by Percentages, in respect to Length, of 200 Individuals of *T. brucei*, Zululand Strain, 1896.



* "Proc." Roy. Soc., B, vol. 83 (1910).

Now, although too much weight must not be placed on this comparison, still it must be confessed that the two curves are remarkably alike, and afford a strong argument that Shilston recovered from the Somkele District of Zululand the same species of trypanosome which had been discovered there in 1894. Again, when the action of these two strains on various animals is compared the same likeness is seen.

Bruce's 1896 strain killed two horses in 30 and 49 days.† Shilston's 1913 strain killed one horse in 35 days. The former strain killed five dogs in an average of 21 days, the latter four dogs in an average of 19 days.

Taking these various arguments into consideration, it may be assumed that the strain of trypanosome, which forms the subject of this paper, belongs to the species *T. brucei*, a well-marked dimorphic type of trypanosome.

The object of this paper is to describe as fully as possible the morphology of this new strain of *T. brucei* from Zululand, in order to try to prove its identity with the trypanosome causing disease in man in Northern and Southern Rhodesia, Nyasaland, and German and Portuguese East Africa. The importance of this cannot be overrated. It has been the habit in the past to consider *T. brucei* harmless to man, but if the above conjecture proves to be true, then all *Glossina morsitans* areas where wild game and *T. brucei* co-exist must be looked upon as dangerous. Evidence is accumulating that this is so. Recently, two Europeans have fallen victims to the tsetse-fly disease in the Sebungwe District in Southern Rhodesia, a remote, savage, unfrequented spot swarming with game and *G. morsitans*. This year also—1913—as had been anticipated, several cases have been found in the Nyasaland fly-areas to the north and south of the Proclaimed Area, one case occurring in a native village within a few miles of Zomba, the official capital.

In future papers, the Susceptibility of Animals to this Strain, its Development in *G. morsitans*, Sera Reactions and Cross Inoculation Experiments will be dealt with.

Morphology of T. brucei, Zululand Strain (No. XXV.), 1913.

A. *Living, Unstained.*

In the living and unstained preparations the dimorphic characteristics of this species can be readily made out. The parasites are actively motile but with restricted translatory movement.

B. *Fixed and Stained.*

The blood films were fixed, stained and measured as previously described in the "Proceedings" of the Royal Society.*

Length.—The following table gives the length of this trypanosome as found in the monkey, dog, guinea-pig and rat, 1,000 trypanosomes in all.

† "Further Report on the Tsetse-fly Disease, or Nagana, in Zululand," 1896.

* B, vol. 81 pp. 16 and 17 (1909).

Table XLIX.—Measurements of the Length of *T. brucei*, Zululand, 1913, Strain XXV.

Date.	No. of Expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913							
Feb. 10	1833	Monkey	Osmic acid	Giemsa	21·4	27·0	16·0
" 10	1835	"	"	"	23·2	29·0	18·0
" 10	1836	"	"	"	23·4	30·0	18·0
" 10	1837	"	"	"	21·9	31·0	17·0
" 13	1834	"	"	"	20·3	28·0	16·0
" 13	1835	"	"	"	19·0	24·0	12·0
" 13	1836	"	"	"	20·1	28·0	16·0
" 17	1834	"	"	"	20·6	27·0	17·0
" 20	1904	Dog	"	"	22·8	29·0	19·0
" 20	1905	"	"	"	20·5	29·0	17·0
" 20	1907	"	"	"	20·6	28·0	17·0
" 20	1908	"	"	"	20·7	30·0	18·0
" 24	1904	"	"	"	25·1	31·0	19·0
" 24	1905	"	"	"	21·1	25·0	18·0
" 24	1907	"	"	"	20·0	30·0	16·0
" 24	1908	"	"	"	21·2	25·0	18·0
" 27	1904	"	"	"	21·7	30·0	18·0
" 27	1905	"	"	"	21·8	31·0	18·0
" 27	1906	"	"	"	20·8	28·0	18·0
" 27	1907	"	"	"	19·1	21·0	16·0
" 27	1908	"	"	"	24·8	32·0	18·0
" 24	1844	Guinea-pig	"	"	27·6	35·0	18·0
" 24	1894	"	"	"	21·0	29·0	18·0
" 27	1843	"	"	"	21·9	29·0	17·0
" 27	1894	"	"	"	21·2	31·0	17·0
" 8	1829	Rat	"	"	22·9	28·0	17·0
" 8	1829	"	"	"	22·5	28·0	18·0
" 8	1829	"	"	"	21·5	26·0	18·0
" 9	1829	"	"	"	21·6	25·0	17·0
" 9	1829	"	"	"	20·9	25·0	18·0
" 9	1829	"	"	"	20·7	24·0	17·0
" 10	1829	"	"	"	21·2	24·0	19·0
" 10	1829	"	"	"	21·6	23·0	19·0
" 10	1829	"	"	"	21·3	24·0	17·0
" 11	1829	"	"	"	19·8	25·0	17·0
" 11	1829	"	"	"	20·5	22·0	18·0
" 11	1829	"	"	"	20·9	25·0	18·0
" 12	1829	"	"	"	20·6	23·0	18·0
" 12	1829	"	"	"	21·7	28·0	18·0
" 12	1829	"	"	"	21·3	25·0	19·0
" 13	1829	"	"	"	20·5	23·0	18·0
" 13	1829	"	"	"	20·0	24·0	17·0
" 13	1829	"	"	"	20·2	23·0	17·0
" 14	1829	"	"	"	20·6	26·0	18·0
" 14	1829	"	"	"	19·5	23·0	17·0
" 14	1829	"	"	"	20·0	22·0	17·0
" 15	1829	"	"	"	20·4	23·0	18·0
" 15	1829	"	"	"	19·7	24·0	17·0
" 15	1829	"	"	"	20·2	22·0	18·0
" 16	1829	"	"	"	19·6	22·0	18·0
					21·0	35·0	12·0

The average length of *T. brucei*, Zululand Strain XXV., 1913, in the monkey, dog, guinea-pig, and rat, taken from Table XLIX., is as follows:—

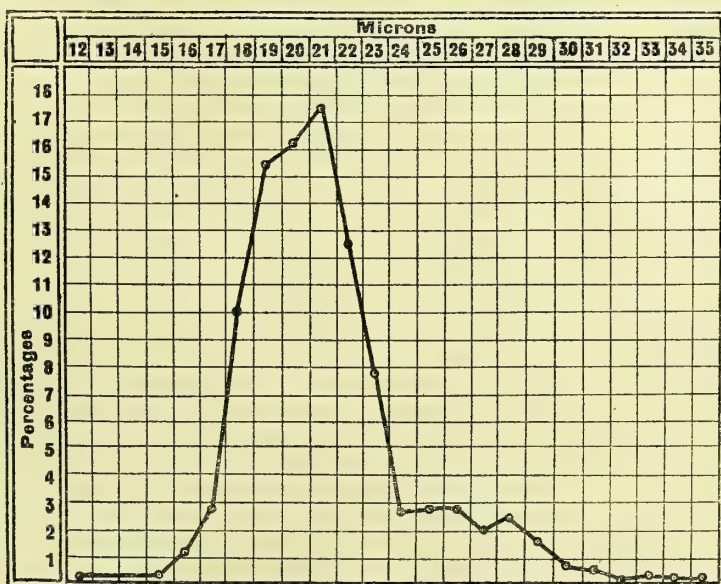
Table L.—Average Length of *T. brucei*, Zululand Strain XXV., 1913.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Monkey	160	21·2	31·0	12·0
Dog	260	21·5	32·0	16·0
Guinea-pig	30	22·9	35·0	17·0
Rat	500	20·8	28·0	17·0

The above table shows that a good deal of difference in growth takes place in different animals. Compare, for example, the guinea-pig with the rat: the former with a maximum of 35 microns, the latter with a maximum of only 28 microns.

The next table gives in detail the distribution in respect to length of 1,000 trypanosomes. The Commission feel hardly justified in taking up space for this purpose, but it is thought that perhaps in some unknown way these figures may be of use to the statistician.

CHART 17.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,000 Individuals of *T. brucei*, Zululand Strain XXV., 1913.



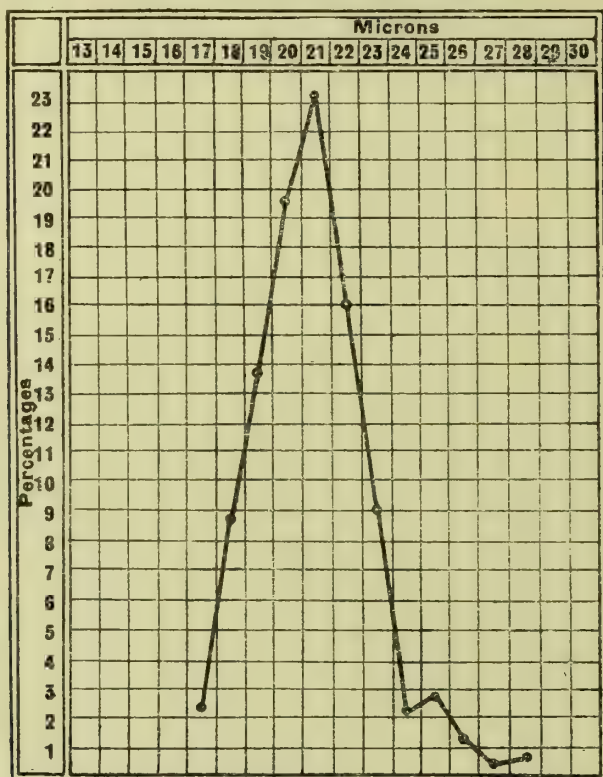
This curve is made up of measurements from 160 specimens of trypanosomes taken from the monkey, 260 from the dog, 80 from the guinea-pig, and 500 from the rat. It is very similar to some of the curves taken from the Nyasaland human strain*: compare Strains II. and VIII. But, on the other hand, it is very unlike some of the others, as for example Strains I. and III.

* "Proceedings" of the Royal Society, B, vol. 86, 1913, p. 394, and vol. 88, 1914, p. 190. (See also Reports of the Sleeping Sickness Commission, No. XV., p. 31; and this Report, p. 49.)

Table LI.—Distribution in respect to Length of 500 Individuals of *T. brucei*, Zululand Strain XXV., 1913.

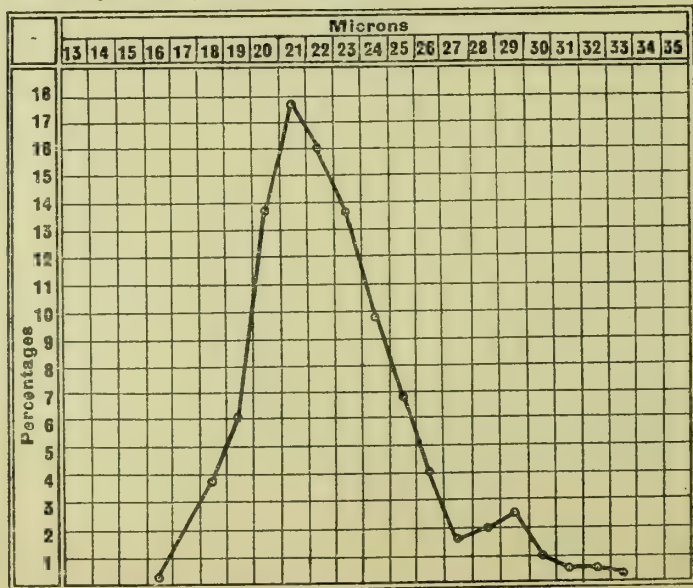
Animal.	In microns.														Average length.										
	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.		26.	27.	28.	29.	30.	31.	32.	33.	34.	35.
Monkey ...	...	—	—	—	1	—	4	2	2	1	2	4	2	2	—	1	1	—	—	—	—	—	—	—	21.4
" "	...	—	—	—	—	—	1	2	2	1	1	1	1	1	1	1	1	—	—	—	—	—	—	—	23.2
" "	...	—	—	—	—	—	2	2	2	1	3	1	1	3	5	—	—	1	—	—	—	—	—	—	23.4
" "	...	—	—	—	4	—	1	1	2	1	4	1	—	1	1	—	—	—	—	1	—	—	—	—	21.9
" "	...	1	—	2	1	1	1	2	3	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	20.3
" "	...	—	—	—	3	2	2	5	4	5	1	—	—	2	—	1	1	—	—	—	—	—	—	—	19.0
" "	...	—	—	—	—	—	—	2	2	5	1	5	1	1	1	2	—	2	—	—	—	—	—	—	20.6
Dog ...	...	—	—	—	—	4	5	3	1	6	1	2	—	—	2	—	1	1	—	—	—	—	—	—	22.8
" "	...	—	—	—	—	2	3	2	2	2	3	3	—	—	—	1	2	—	—	—	—	—	—	—	20.5
" "	...	—	—	—	—	—	3	3	2	1	1	1	1	1	1	1	3	1	1	—	—	—	—	—	20.6
" "	...	—	—	—	—	—	—	2	3	5	4	2	1	1	2	2	2	3	1	1	—	—	—	—	20.7
" "	...	—	—	—	1	—	—	—	3	2	3	1	—	—	—	—	—	—	—	—	—	—	—	—	25.1
" "	...	—	—	—	—	—	2	2	4	2	4	2	1	1	—	—	—	—	1	—	—	—	—	—	21.1
" "	...	—	—	—	—	1	2	4	2	2	3	1	—	2	—	—	—	—	—	—	—	—	—	—	20.0
" "	...	—	—	—	—	—	—	3	4	3	2	6	—	—	1	1	1	—	—	—	—	—	—	—	21.2
" "	...	—	—	—	—	—	—	4	6	1	1	—	2	2	1	1	1	1	1	—	—	—	—	—	21.7
" "	...	—	—	—	—	—	—	2	3	2	2	—	—	—	—	2	1	—	1	1	—	—	—	—	21.8
" "	...	—	—	—	—	—	1	3	6	3	4	—	3	—	1	—	1	—	—	—	—	—	—	—	20.8
" "	...	—	—	—	1	1	4	6	5	3	—	—	—	—	—	1	—	—	—	—	—	—	—	—	19.1
" "	...	—	—	—	—	—	2	3	2	—	1	—	—	1	2	1	1	3	2	1	1	1	—	—	24.8
Guinea-pig	...	—	—	—	—	—	1	1	—	—	—	2	1	—	1	1	4	2	1	2	—	2	1	—	27.6
" "	...	—	—	—	—	—	2	5	1	4	—	3	—	—	—	1	1	2	—	—	—	—	—	—	21.0
" "	...	—	—	—	—	—	5	2	2	2	2	—	—	—	4	1	1	2	—	—	—	—	—	—	21.9
" "	...	—	—	—	—	—	4	5	1	2	1	—	1	1	—	—	1	1	1	—	—	—	—	—	21.2

CHART 18.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals, of *T. brucei*, Zululand Strain XXV., 1913, taken on 9 consecutive days from Rat 1829.



This is rather a peculiar curve, but is not unlike curves obtained in a similar way from the trypanosome causing disease in man in Nyasaland, as the following chart will show:—

CHART 19.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome causing Disease in Man in Nyasaland, taken on 9 consecutive days from Rat 2300.



The Zululand Strain XXV., 1913, has been carried on in horses and cattle for some time in the laboratory at Pretoria, and may have varied somewhat in morphology under these artificial conditions. It will be interesting to see what change, if any, is induced by passage through *G. morsitans*. The three following curves represent first, second, and third passages:—

CHART 20.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. brucei*, Zululand Strain XXV., 1913, after first passage through *G. morsitans*, taken on 9 consecutive days from Rat 2006.

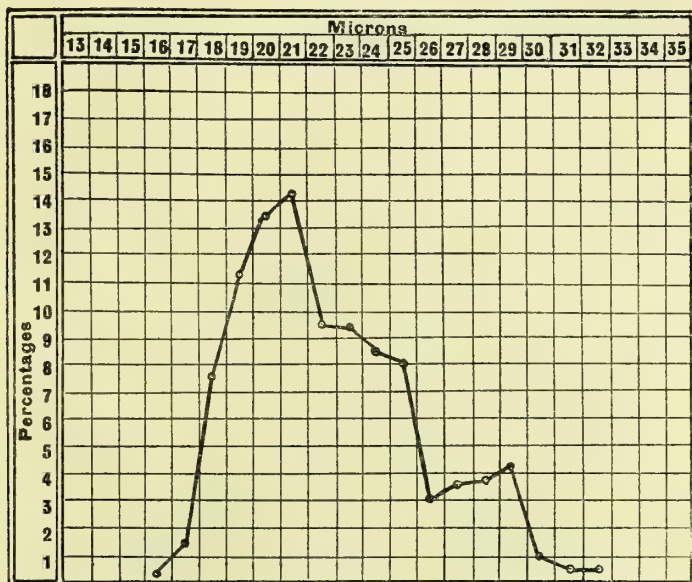


CHART 21.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. brucei*, Zululand Strain XXV., 1913, after second passage through *G. morsitans*, taken on 9 consecutive days from Rat 2288.

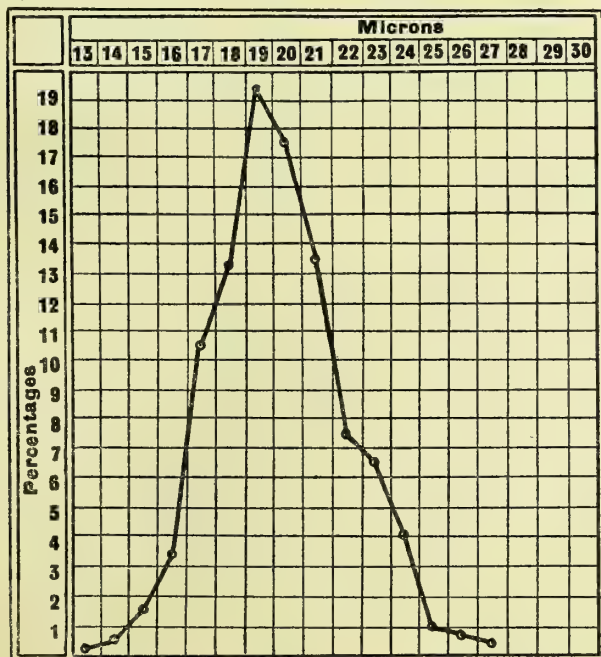
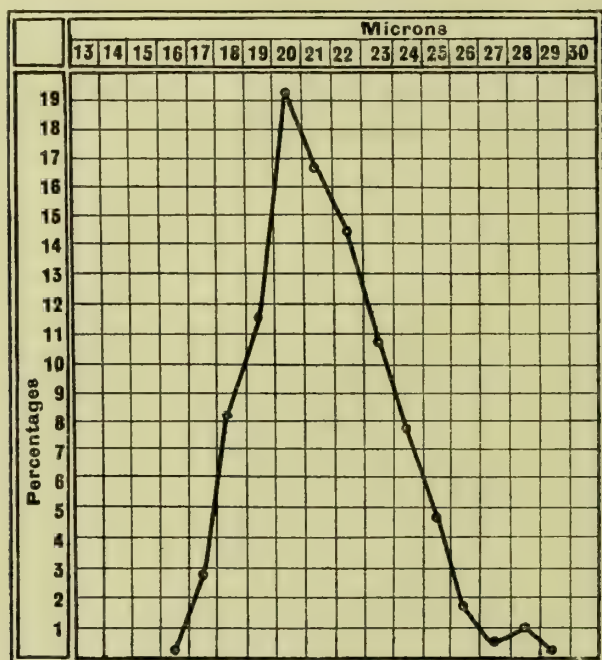


CHART 22.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. brucei*, Zululand Strain XXV., 1913, after third passage through *G. morsitans*, taken on 9 consecutive days from Rat 2406.



From this curve it will be seen that three passages through *G. morsitans* has had little or no effect in changing the character of this trypanosome as regards distribution of length. It has usually been thought that a trypanosome kept under laboratory conditions, and without the opportunity of passage through its invertebrate host, the tsetse fly, would tend to change in morphology. These curves, on the other hand, show that a trypanosome, after passage through horses and cattle for some years—exact time unknown—is unchanged by three passages through its invertebrate host, *G. morsitans*.

Table LII.—Distribution in respect to Length of 500 Individuals of *T. brucei*, Zululand Strain XXV., 1913, after first passage through *G. morsitans*.

Animal.	In microns.																	Average length.
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	
Rat ...	—	1	1	5	—	2	1	1	4	1	—	2	—	2	—	—	—	22.5
" ...	—	—	—	1	3	—	4	3	1	3	—	1	3	—	1	—	—	23.8
" ...	—	1	3	—	1	4	2	4	1	1	1	—	1	1	—	—	—	22.1
" ...	—	—	3	—	5	2	3	3	1	2	—	—	—	1	—	—	—	21.7
" ...	—	1	1	1	2	6	3	1	4	—	—	—	1	—	—	—	—	21.4
" ...	—	—	2	—	3	6	4	2	2	1	—	—	—	—	—	—	—	21.4
" ...	—	—	—	3	4	4	2	1	1	1	—	1	1	—	—	—	1	22.3
" ...	—	—	3	1	8	3	—	1	1	2	—	1	—	—	—	—	—	21.0
" ...	—	—	3	3	5	1	2	2	—	—	—	2	—	2	—	—	—	21.7
" ...	—	—	2	2	1	—	1	2	2	1	1	—	2	4	2	—	—	24.6
" ...	—	—	—	3	3	1	1	—	2	4	1	—	—	2	2	—	1	24.1
" ...	—	—	1	2	—	1	1	—	3	4	4	1	1	2	—	—	—	24.0
" ...	—	—	2	4	1	1	4	—	2	3	—	2	—	—	1	—	—	22.4
" ...	—	—	1	1	1	1	2	2	5	3	2	—	—	1	—	1	—	23.7
" ...	—	—	1	1	1	2	2	3	3	3	2	1	—	1	—	—	—	23.3
" ...	—	2	2	2	1	3	1	3	2	1	—	2	—	1	—	—	—	21.9
" ...	—	1	3	1	2	3	4	—	—	4	—	—	2	—	—	—	—	21.8
" ...	—	—	2	3	2	5	1	5	—	1	—	1	—	—	—	—	—	21.3
" ...	—	—	1	5	4	5	1	—	—	—	2	1	—	1	—	—	—	21.4
" ...	1	—	1	1	3	3	2	4	1	1	—	—	2	1	—	—	—	22.3
" ...	—	—	1	5	1	4	2	1	3	—	1	—	1	1	—	—	—	21.9
" ...	—	—	1	4	5	4	1	2	—	1	—	1	1	—	—	—	—	21.3
" ...	—	—	1	2	3	5	2	2	1	1	1	—	1	1	—	—	—	22.1
" ...	—	—	—	1	4	4	3	1	2	2	1	—	1	—	1	—	—	22.7
" ...	—	1	3	5	4	1	—	—	1	—	2	1	2	—	—	—	—	21.3
Total ...	1	7	38	56	67	71	48	47	43	40	15	18	19	21	5	2	2	
Per- centages.	0.2	1.4	7.6	11.2	13.4	14.2	9.6	9.4	8.6	8.0	3.0	3.6	3.8	4.2	1.0	0.4	0.4	

Table LIII.—Distribution in respect to Length of 500 Individuals of *T. brucei*, Zululand Strain XXV., 1913, after second passage through *G. morsitans*.

Animal.	In microns.																	Average length.
	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.			
Rat	—	—	—	—	1	2	—	5	4	2	3	2	1	—	—	21.1		
"	—	—	—	—	2	—	4	3	3	1	4	2	—	1	—	21.0		
"	—	—	—	—	1	2	2	3	2	1	2	4	1	2	—	21.7		
"	—	—	—	—	1	3	4	5	4	2	1	—	—	—	—	19.9		
"	—	—	—	—	2	2	9	3	3	1	—	—	—	—	—	19.3		
"	—	—	1	—	3	2	5	5	4	—	—	—	—	—	—	19.0		
"	—	—	1	3	4	—	5	1	2	1	1	1	1	—	—	19.1		
"	—	—	1	—	5	2	2	4	4	1	—	—	—	—	1	19.3		
"	—	—	—	2	—	2	5	3	2	4	2	—	—	—	—	19.9		
"	—	—	—	2	1	3	3	5	3	—	2	1	—	—	—	19.6		
"	—	—	1	—	2	3	5	5	3	—	—	—	—	—	1	19.4		
"	—	—	—	1	3	4	1	3	4	2	1	—	—	1	—	19.7		

Table LIII. (continued).

Animal.	In microns.																Average length.
	13.	14.	15.	16.	17.	18.	18.	20.	21.	22.	23.	24.	25.	26.	27.		
Rat	—	2	1	3	4	3	2	2	2	1	—	—	—	—	—	17.7	
"	1	—	—	1	2	4	6	3	—	—	3	—	—	—	—	18.9	
"	—	—	1	1	6	5	3	3	—	—	1	—	—	—	—	18.1	
"	—	—	—	2	3	5	2	3	1	2	1	1	—	—	—	19.1	
"	—	—	—	—	2	1	7	5	4	1	—	—	—	—	—	19.5	
"	—	—	—	—	4	3	4	4	3	—	1	1	—	—	—	19.4	
"	—	—	1	1	3	3	3	2	5	1	1	—	—	—	—	19.1	
"	—	—	1	—	—	2	5	5	—	6	1	—	—	—	—	20.0	
"	—	—	—	—	—	1	7	5	3	1	1	2	—	—	—	20.3	
"	—	—	—	—	1	2	4	5	2	2	2	2	—	—	—	20.4	
"	—	—	—	—	2	2	2	1	4	5	3	—	1	—	—	20.8	
"	—	—	—	—	1	3	2	2	3	3	1	4	1	—	—	21.1	
"	—	—	—	1	—	7	5	3	2	—	2	—	—	—	—	19.2	
Total	1	2	8	17	53	66	97	88	67	37	33	20	5	4	2		
Percentages	0.2	0.4	1.6	3.4	10.6	13.2	19.4	17.6	13.4	7.4	6.6	4.0	1.0	0.8	0.4		

Table LIV.—Distribution in respect to Length of 500 Individuals of *T. brucei*, Zululand Strain XXV., 1913, after third passage through *G. morsitans*.

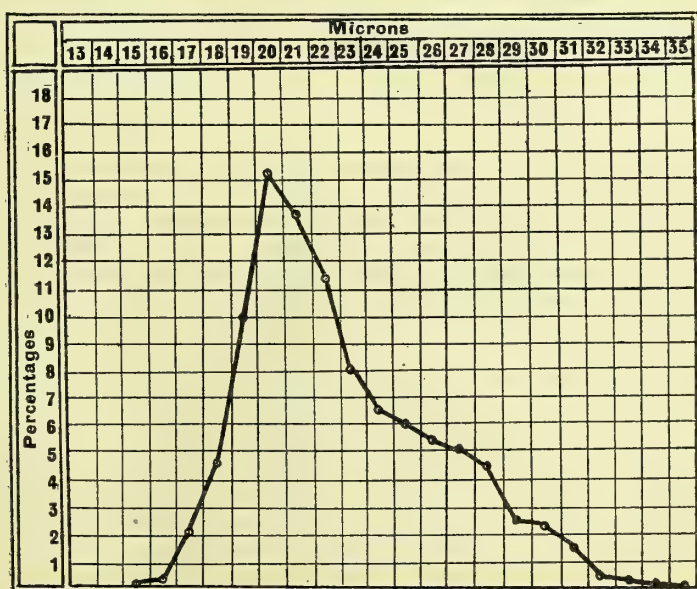
Animal.	In microns.															Average length.
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.		
Rat	—	—	1	2	3	2	5	4	2	1	—	—	—	—	21.6	
"	—	—	3	1	3	5	3	—	2	3	—	—	—	—	21.3	
"	—	1	1	—	3	6	3	4	—	2	—	—	—	—	21.4	
"	—	—	2	1	2	6	1	4	2	—	1	—	1	—	21.8	
"	—	—	1	1	1	3	3	5	4	2	—	—	—	—	22.3	
"	—	—	2	2	5	2	2	3	2	—	1	1	—	—	21.5	
"	—	—	—	3	5	8	2	1	1	—	—	—	—	—	20.8	
"	—	1	1	4	5	2	3	2	1	1	—	—	—	—	20.7	
"	—	1	1	3	2	7	3	1	1	—	—	—	1	—	21.0	
"	—	2	—	3	4	1	5	4	1	—	—	—	—	—	20.8	
"	—	—	1	3	6	4	2	2	2	—	—	—	—	—	20.8	
"	—	—	1	1	3	4	2	5	2	1	1	—	—	—	21.9	
"	—	—	1	4	2	1	1	2	3	5	1	—	—	—	22.3	
"	1	—	3	3	3	—	4	3	—	1	1	—	1	—	21.1	
"	—	—	1	2	2	1	8	1	3	1	—	—	—	1	12.1	
"	—	2	1	1	7	3	2	2	1	1	—	—	—	—	20.6	
"	—	—	2	2	4	6	3	1	—	1	—	1	—	—	21.0	
"	—	—	3	3	8	1	1	—	2	—	1	—	1	—	20.8	
"	—	1	1	5	4	1	2	1	2	2	1	—	—	—	21.1	
"	—	1	3	3	2	4	3	3	1	—	—	—	—	—	20.5	
"	—	1	4	3	5	2	1	2	—	2	—	—	—	—	20.3	
"	—	—	3	1	5	4	4	—	2	—	—	—	1	—	21.0	
"	—	2	1	1	5	2	4	2	1	1	1	—	—	—	21.1	
"	—	2	3	3	1	4	4	1	2	—	—	—	—	—	20.4	
"	—	—	1	3	6	5	1	1	2	—	1	—	—	—	30.9	
Total	1	14	41	58	96	84	72	54	39	24	9	2	5	1		
Percentages	0.2	2.8	8.2	11.6	19.2	16.8	14.4	10.8	7.8	4.8	1.8	0.4	1.0	0.2		

CHART 23.—Curve representing the Distribution, by Percentages, in respect to Length, of 2,000 Individuals of *T. brucei*, Zululand Strain XXV., 1913, taken on 9 consecutive days from Rats 1829, 2006, 2288, and 2406.



As Shilston's mule was in all probability infected from the wild game of the Somkele District, in Zululand, it will be interesting to compare this curve with that of the trypanosome causing disease in man, taken from the wild game in Nyasaland.

CHART 24.—Curve representing the Distribution, by Percentages, in respect to Length, of 2,500 Individuals of the Trypanosome causing Disease in Man in Nyasaland, the Wild-game Strain, taken on 9 consecutive days from Rats 847, 1220, 992, 849, and 1022.



These two curves are undoubtedly much alike, and, as the wild-game strain in Nyasaland is supposed to be identical with the human strain, then it might be said that *T. brucei*, Zululand Strain XXV., 1913, is also identical with the trypanosome causing disease in man in Nyasaland. Others will say that the Zululand trypanosome and the Nyasaland wild-game strain are both *T. brucei*, but that this does not prove that *T. brucei* is identical with the human strain. No, but if it is shown that *T. brucei*, Zululand, is absolutely identical in morphology with this Nyasaland human strain, that it also has exactly the same disease-producing power on the various experimental animals, this will go a good long way to make the ordinary unprejudiced man chary of exposing himself too carelessly to the so-called harmless nagana. His serum may save him as a rule, but there may come a time when—his resistance being lowered either by fatigue or some other cause—the trypanosome may gain a footing, and then his belief in the written word of the text-book will receive a rude shock.

Breadth.—The long and slender average 2.76 microns in breadth, the intermediate 3.25, and the short and stumpy 3.53. This measurement was made across the broadest part of the trypanosome and includes the undulating membrane. Most previous measurements of breadth have not included this.

Table LV.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of *T. brucei*, Zululand Strain XXV., 1913.

Date.					Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.							
June	22...	...	...	...	I.	Horse	30
"	25...	...	...	...	II.	"	65
—	...	...	...	...	I.	Dog	5
June	21...	...	...	...	II.	"	Nil
1913.							
Feb.	8...	...	...	...	1828	Rat	1
"	9	...	...	...	1828	"	37
"	10...	...	...	...	1828	"	63
"	11...	...	...	...	1828	"	36
"	12...	...	...	...	1828	"	32
"	13...	...	...	...	1828	"	17
"	14...	...	...	...	1828	"	54
"	15...	...	...	...	1828	"	45
"	16...	...	...	...	1828	"	20
"	17...	...	...	...	1828	"	17
Mar.	24...	...	...	...	2006	"	10
"	25...	...	...	...	2006	"	54
"	26...	...	...	...	2006	"	41
"	27...	...	...	...	2006	"	18
"	28...	...	...	...	2006	"	38
"	29...	...	...	...	2006	"	32
Apr.	1...	...	...	...	2006	"	69

Table LV. (continued).

Date.					Experiment No.	Animal.	Percentage among short and stumpy forms.
1913.							
Apr.	2...	...	...	...	2006	Rat	74
"	3...	...	...	...	2006	"	63
"	7...	...	...	...	2006	"	74
July	21...	...	...	...	2288	"	Nil
"	22...	...	...	...	2288	"	42
"	23...	...	...	...	2288	"	20
"	24...	...	...	...	2288	"	35
"	25...	...	...	...	2288	"	16
"	26...	...	...	...	2288	"	29
"	27...	...	...	...	2288	"	43
"	28...	...	...	...	2288	"	52
"	29...	...	...	...	2288	"	49
"	30...	...	...	...	2288	"	61
...					I.	Mouse	5
Average ...					...	...	37.8

In regard to the shape of this trypanosome, contents of cell, nucleus, micronucleus, undulating membrane, and flagellum, it is not proposed to describe these characters separately for this strain as was done in the case of the trypanosome causing disease in man in Nyasaland. Suffice it to say that, after the most careful comparison, no difference whatever can be made out in the morphology of the two trypanosomes. At the end of this paper three plates are given, one representing the short and stumpy, another the intermediate, and a third the long and slender forms. If these plates are compared with those given in previous papers* it will be seen that in morphology the Nyasaland and Zululand trypanosomes are identical.

Conclusions.

1. The trypanosome described above under the name of the "Zululand 1913 Strain," is the same species as that discovered by Bruce in Zululand in 1894; reported on by Kanthack, Durham, and Blandford in 1898; and named *T. brucei* by Plimmer and Bradford in 1899.

2. As regards its morphology, this trypanosome is absolutely identical with the trypanosome causing disease in man in Nyasaland, the *T. rhodesiense* of Stephens and Fantham.

* "Proc." Roy. Soc., B, vol. 85, p. 433 (1912), and vol. 87, p. 35 (1913), and Reports of the Sleeping Sickness Commission, No. XV., pp. 10 and 110.

DESCRIPTION OF PLATES.

PLATE 1.

Trypanosoma brucei, Zululand 1913 Strain, short and stumpy forms.
× 2000.

PLATE 2.

Trypanosoma brucei, Zululand 1913 Strain, intermediate forms.
× 2000.

PLATE 3.

Trypanosoma brucei, Zululand 1913 Strain, long and slender forms.
× 2000.

XXI.—MORPHOLOGY OF VARIOUS STRAINS OF THE
TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHO-*
DESIENSE—CAUSING DISEASE IN MAN IN NYASA-
LAND. THE NATURALLY-INFECTED DOG STRAIN.
STRAINS XXVI. TO XXVIII.*

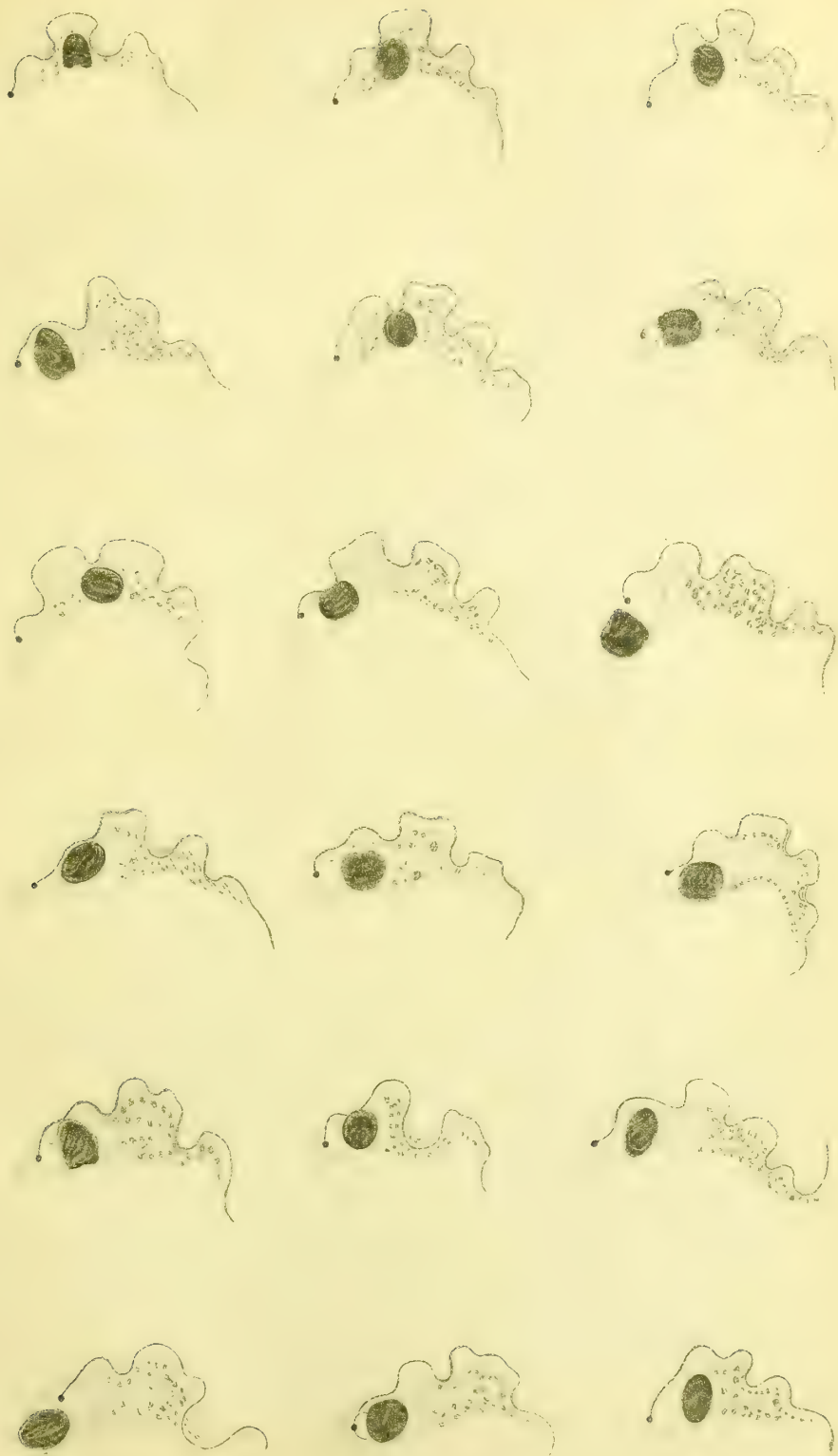
This strain differs so much from the others, that it is doubtful if it should be included among the various strains already described—Human, Wild Game, Wild *Glossina morsitans*, Mzimba, etc. It has only been found on three occasions, and, curiously enough, each time in a native dog.

The three dogs suffering from trypanosome disease were brought up to Kasu from the Sleeping Sickness Area, where they had probably been naturally-infected by the wild *Glossina morsitans*, hence the name, the Naturally-Infected Dog Strain.

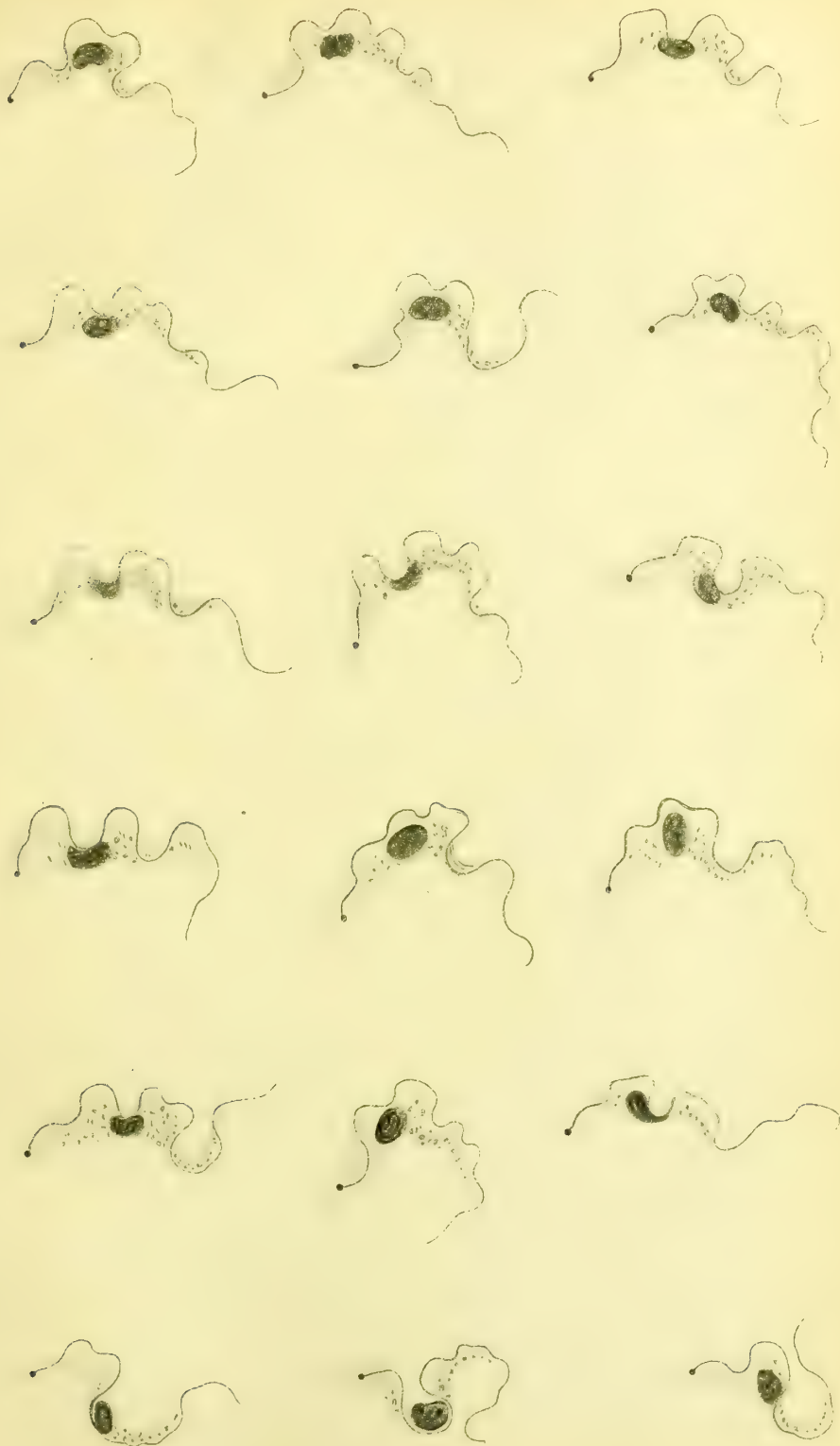
All the infected dogs coming from this area did not show this strain; for example, Dog 553 was infected with a trypanosome resembling the ordinary Human Strain.

If this Naturally-Infected Dog Strain had been found in the blood of the wild game and in the wild *Glossina morsitans*, then it would have been legitimate to make a new species of it. But it would be unjustifiable to make a new species of a strain which, up to the present, has only been found in three chronically-infected dogs. The Commission have, therefore, decided to consider this strain as belonging to the species described as the Trypanosome causing Disease in Man in Nyasaland—*Trypanosoma brucei* vel *rhodesiense*—and not as a new species. If this is correct, then it is curious how much a species can vary in disease-producing power. For example, it will be shown that this Naturally-Infected Dog Strain is almost harmless to monkeys and guinea-pigs, whereas the parent species kills these animals without fail. Not only does it differ in virulence, but even its morphology is apparently somewhat changed. There is a comparative absence of the blunt-ended posterior-nucleated forms, which are sometimes so marked a feature in the parent species. Not that they are altogether absent, but they are not so prominent, do not strike the eye so readily. It will, therefore, be interesting to describe this strain as fully and completely as possible.

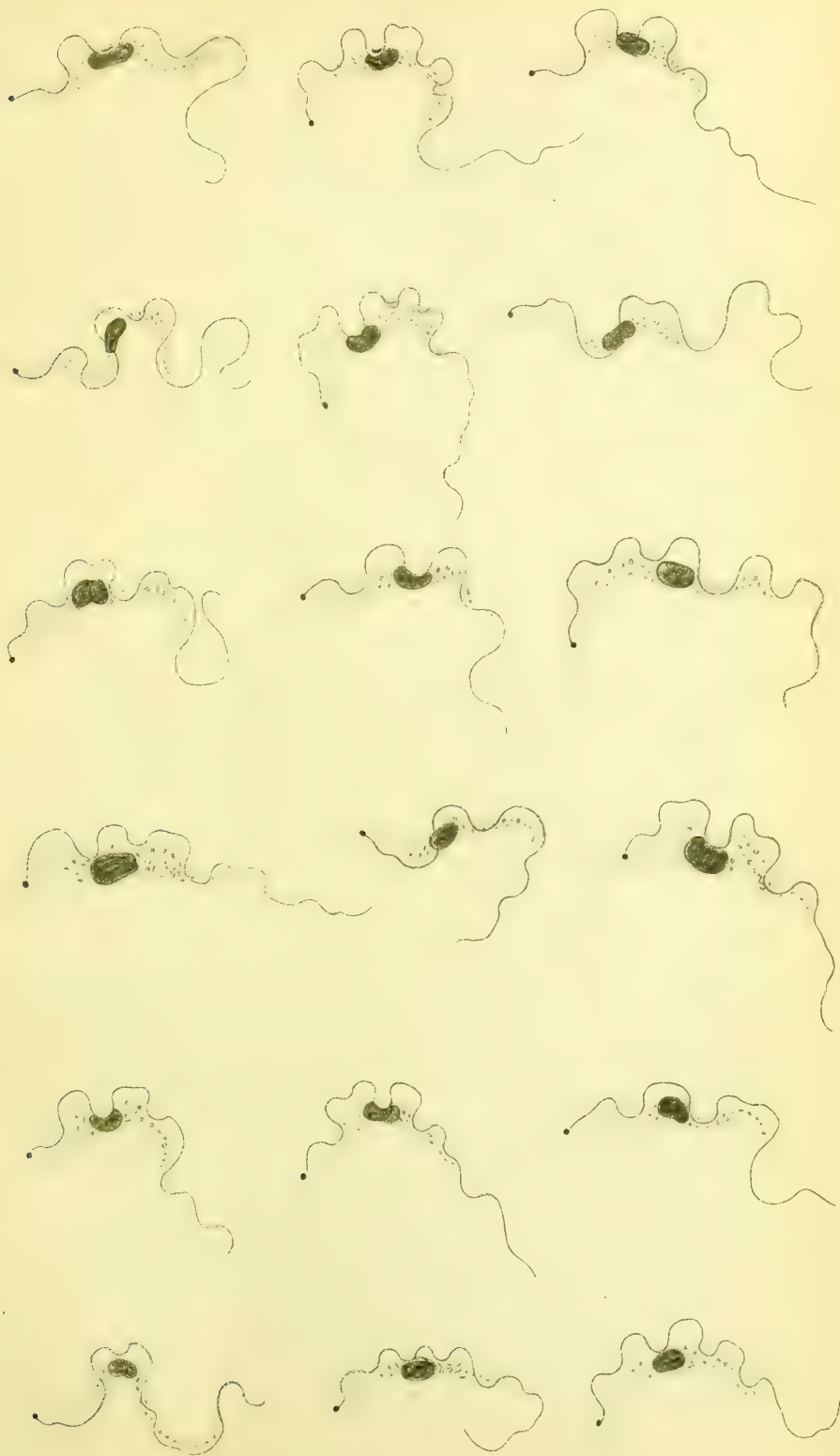
* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 111.



Short & Stumpy.



Intermediate.



Long & Slender.

*Morphology of the Naturally-Infected Dog Strain.
Strain XXVI., from Dog 48.*

Table LVI.—Measurements of the Length of the Trypanosome of Naturally-Infected Dog. Strain XXVI., from Dog 48.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Feb. 17	191	Ox	Osmic acid	Giemsa	25.5	31.0	16.0
" 22	196	Sheep	"	"	26.4	30.0	21.0
Jan. 20	48	Dog	"	"	23.6	29.0	19.0
" 24	48	"	"	"	24.2	31.0	16.0
Feb. 1	48	"	"	"	25.7	35.0	15.0
" 15	140	"	"	"	24.5	30.0	17.0
" 19	139	"	"	"	29.8	23.0	26.0
" 19	139	"	"	"	32.3	35.0	25.0
" 22	69	"	"	"	30.1	33.0	26.0
" 26	151	"	"	"	28.5	32.0	23.0
" 28	210	"	"	"	22.8	32.0	19.0
" 29	210	"	"	"	20.1	23.0	18.0
Apl. 15	317	"	"	"	20.1	31.0	18.0
" 19	317	"	"	"	23.2	31.0	19.0
" 22	331	"	"	"	22.6	31.0	16.0
" 8	389	Rabbit	"	"	22.9	29.0	15.0
" 15	389	"	"	"	26.0	29.0	18.0
" 15	390	"	"	"	22.3	35.0	15.0
" 16	389	"	"	"	24.3	30.0	17.0
Feb. 13	67	Rat	"	"	21.4	28.0	18.0
" 13	67	"	"	"	20.9	31.0	17.0
" 13	67	"	"	"	22.8	31.0	18.0
" 15	67	"	"	"	19.8	30.0	17.0
" 15	189	"	"	"	24.9	30.0	18.0
" 15	190	"	"	"	25.6	29.0	17.0
" 19	189	"	"	"	28.6	35.0	21.0
" 22	190	"	"	"	28.0	34.0	18.0
" 26	67	"	"	"	23.2	33.0	18.0
" 29	67	"	"	"	20.5	31.0	16.0
Mar. 11	67	"	"	"	20.7	30.0	16.0
Apr. 3	312	"	"	"	26.6	32.0	18.0
" 5	312	"	"	"	21.7	32.0	18.0
" 8	391	"	"	"	20.3	30.0	16.0
" 8	392	"	"	"	24.8	32.0	16.0
" 8	392	"	"	"	25.8	32.0	17.0
" 8	392	"	"	"	25.5	32.0	16.0
" 11	311	"	"	"	29.5	35.0	19.0
" 11	312	"	"	"	25.4	32.0	16.0
" 12	311	"	"	"	20.8	31.0	15.0
" 12	312	"	"	"	23.9	36.0	19.0
" 12	391	"	"	"	27.6	32.0	19.0
" 12	392	"	"	"	24.3	30.0	18.0
" 13	311	"	"	"	21.9	32.0	18.0
" 13	312	"	"	"	21.7	33.0	19.0
" 13	391	"	"	"	29.3	35.0	19.9
" 13	392	"	"	"	23.7	36.0	11.0
" 14	407	"	"	"	23.0	33.0	16.0
" 15	312	"	"	"	22.1	33.0	18.0
" 15	291	"	"	"	25.0	33.0	19.0
" 15	392	"	"	"	21.8	35.0	19.0
" 16	312	"	"	"	21.8	32.0	17.0
" 16	291	"	"	"	19.7	28.0	17.0
					24.2	36.0	15.0

Table LVII.—Measurements of the Length of 500 Specimens of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, taken on 9 consecutive days from Rat 1218, after passage through rats for seven months.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Sept. 5	1218	Rat	Osmic acid	Giemsa	25·8	31·0	17·0
" 5	1218	"	"	"	27·3	33·0	19·0
" 5	1218	"	"	"	25·1	31·0	18·0
" 6	1218	"	"	"	23·0	31·0	16·0
" 6	1318	"	"	"	21·7	32·0	16·0
" 6	1218	"	"	"	23·7	31·0	18·0
" 7	1218	"	"	"	26·4	30·0	21·0
" 7	1218	"	"	"	26·0	30·0	20·0
" 7	1218	"	"	"	25·2	31·0	18·0
" 8	1218	"	"	"	26·2	32·0	21·0
" 8	1218	"	"	"	25·3	31·0	18·0
" 8	1218	"	"	"	26·0	30·0	19·0
" 9	1218	"	"	"	28·1	32·0	24·0
" 9	1218	"	"	"	27·8	32·0	21·0
" 9	1218	"	"	"	27·0	31·0	21·0
" 19	1218	"	"	"	22·6	31·0	17·0
" 10	1218	"	"	"	20·8	30·0	16·0
" 10	1218	"	"	"	22·9	32·0	18·0
" 11	1218	"	"	"	21·2	29·0	18·0
" 11	1218	"	"	"	20·4	23·0	17·0
" 11	1218	"	"	"	20·9	26·0	18·0
" 12	1218	"	"	"	23·0	33·0	18·0
" 12	1218	"	"	"	20·1	23·0	17·0
" 12	1218	"	"	"	23·2	32·0	16·0
" 13	1218	"	"	"	21·9	29·0	19·0
					24·1	33·0	16·0

Table LVIII.—Measurements of the Length of 500 Specimens of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, taken on 9 consecutive days from Rat 2471, after passages through rats for two years. Series of 46 animals.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Dec. 26	2471	Rat	Osmic acid	Giemsa	29·5	34·0	22·0
" 26	2471	"	"	"	28·6	32·0	25·0
" 26	2471	"	"	"	29·1	33·0	25·0
" 27	2471	"	"	"	28·4	31·0	26·0
" 27	2471	"	"	"	29·0	31·0	26·0
" 27	2471	"	"	"	28·0	33·0	19·0
" 28	2471	"	"	"	30·3	35·0	20·0
" 28	2471	"	"	"	31·0	35·0	27·0
" 28	2471	"	"	"	29·8	36·0	22·0

Table LVIII. (*continued*).

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1914.							
Jan. 1	2471	Rat	Osmic acid	Giemsa	28·7	32·0	24·0
" 1	2471	"	"	"	28·7	31·0	25·0
" 1	2471	"	"	"	27·8	30·0	19·0
" 3	2471	"	"	"	29·3	33·0	23·0
" 3	2471	"	"	"	30·4	34·0	25·0
" 3	2471	"	"	"	30·4	34·0	26·0
" 4	2471	"	"	"	26·8	33·0	20·0
" 4	2471	"	"	"	27·6	32·0	19·0
" 4	2471	"	"	"	27·9	32·0	19·0
" 5	2471	"	"	"	28·2	33·0	20·0
" 5	2471	"	"	"	26·6	32·0	18·0
" 5	2471	"	"	"	27·3	33·0	19·0
" 6	2471	"	"	"	27·9	32·0	19·0
" 6	2471	"	"	"	28·0	33·0	24·0
" 6	2471	"	"	"	28·7	33·0	20·0
" 7	2471	"	"	"	26·9	32·0	19·0
					28·6	36·0	18·0

The average length of the trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, taken from Tables LVI., LVII., and LVIII., is as follows:—

Table LIX.—Average Length of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48.

Species of animal.				Number of trypanosomes measured.	In microns.		
					Average length.	Maximum length.	Minimum length.
Ox ...	...	...	...	20	25·5	31·0	16·0
Sheep ...	...	...	...	20	26·4	30·0	21·9
Dog ...	...	...	...	260	25·2	35·0	15·0
Rabbit ...	...	...	...	80	23·9	35·0	15·0
Rat ...	...	...	...	660	23·7	36·0	15·0

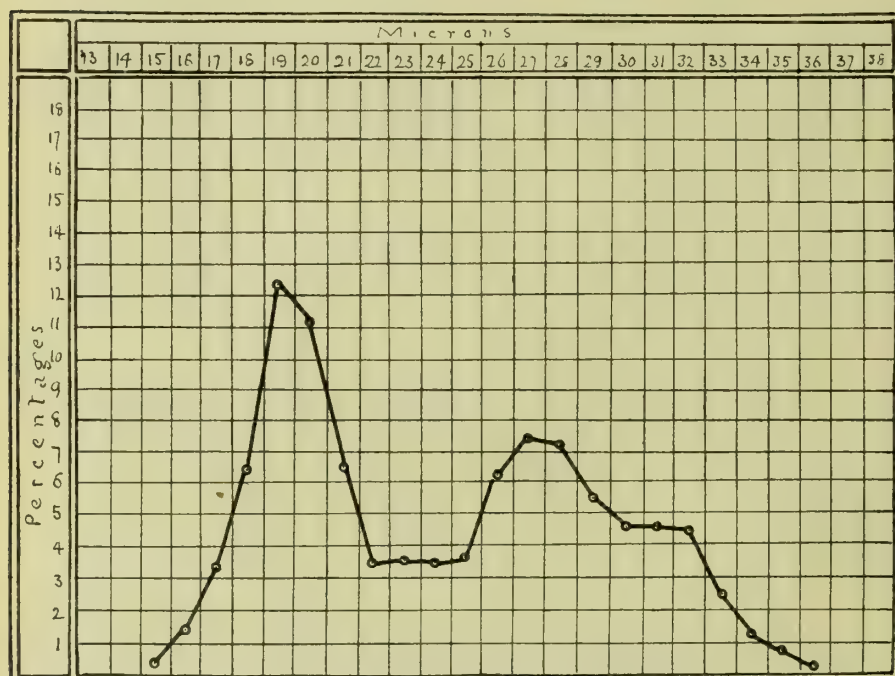
Table LX.—Average Length of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, after passage through rats for 7 months.

Species of animal.				Number of trypanosomes measured.	In microns.		
					Average length.	Maximum length.	Minimum length.
Rat ...	...	...	...	500	24·1	33·0	19·0

Table LXI.—Average Length of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, after passage through rats, for 2 years. Series of 46 animals.

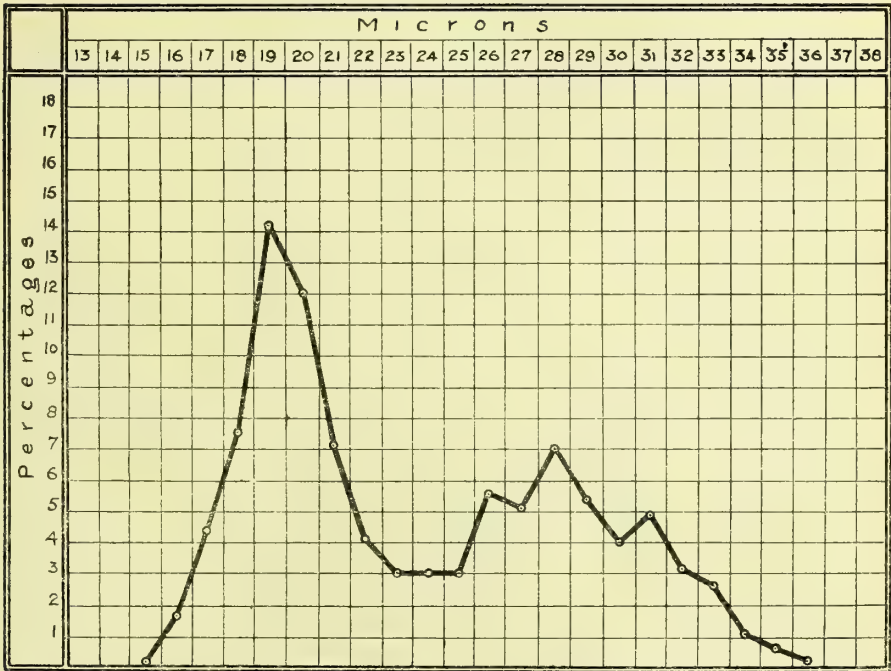
Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Rat	500	28.6	36.0	18.0

CHART 25.—Curve representing the Distribution, by Percentages, in respect to Length, of 1,040 Individuals of the Trypanosome of Naturally-Infected Dog, Strain XXVI. from Dog 48, taken at random from various animals.



This curve is made up of measurements from 20 specimens of trypanosomes taken from the ox, 20 from the sheep, 260 from the dog, 80 from the rabbit, and 660 from the rat.

CHART 26.—Curve representing the Distribution, by Percentages, in respect to Length, of 660 Individuals of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, taken at random from several rats.

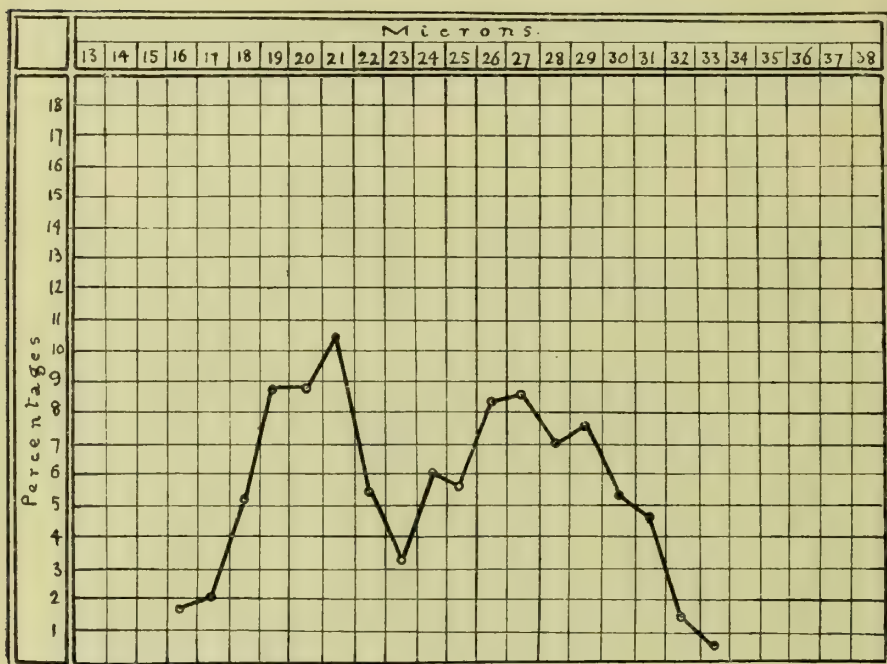


This is the curve of a markedly dimorphic type, and may be compared with the Wild Game Strain I.,* or with the Wild *Glossina morsitans* Strains IV. and V.† The above curve shows the strain as it appeared in the rat in February, 1912, when it was first obtained. The next curve shows the same strain as it appeared in the rat in September, 1912, after it had passed for seven months through a series of eight rats.

* "Proc." Roy. Soc., B, vol. 86, 1913, and Reports of the Sleeping Sickness Commission, No. XXV., p. 48.

† "Proc." Roy. Soc., B, vol. 86, 1913, pp. 415 and 417, and Reports of the Sleeping Sickness Commission, No. XV., p. 61.

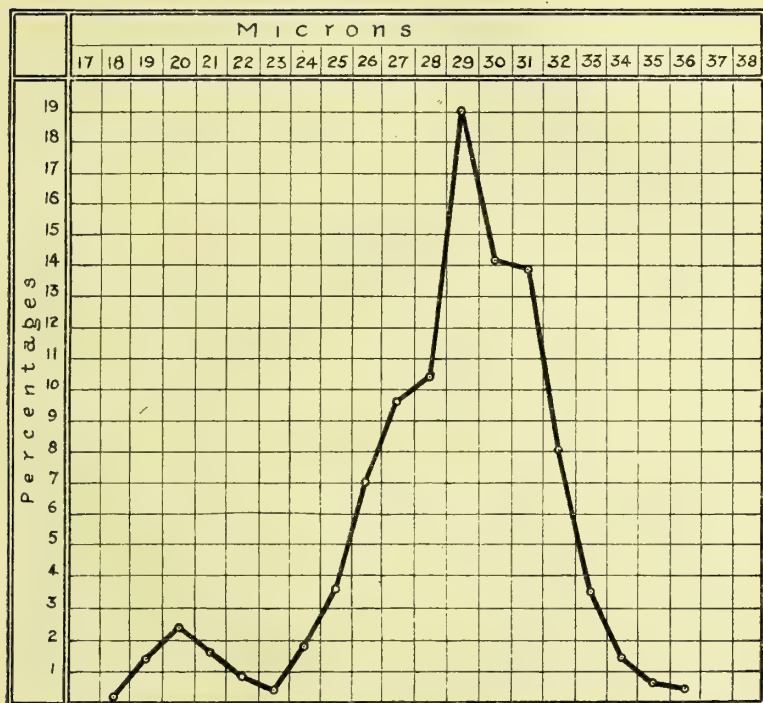
CHART 27.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, taken on 9 consecutive days from Rat 1218, after passing through a series of 8 rats.



This curve still shows a markedly dimorphic type, but the proportion of the long forms is increasing.

The next curve shows the same strain at the beginning of 1914, after passing for two years through a series of 46 rats.

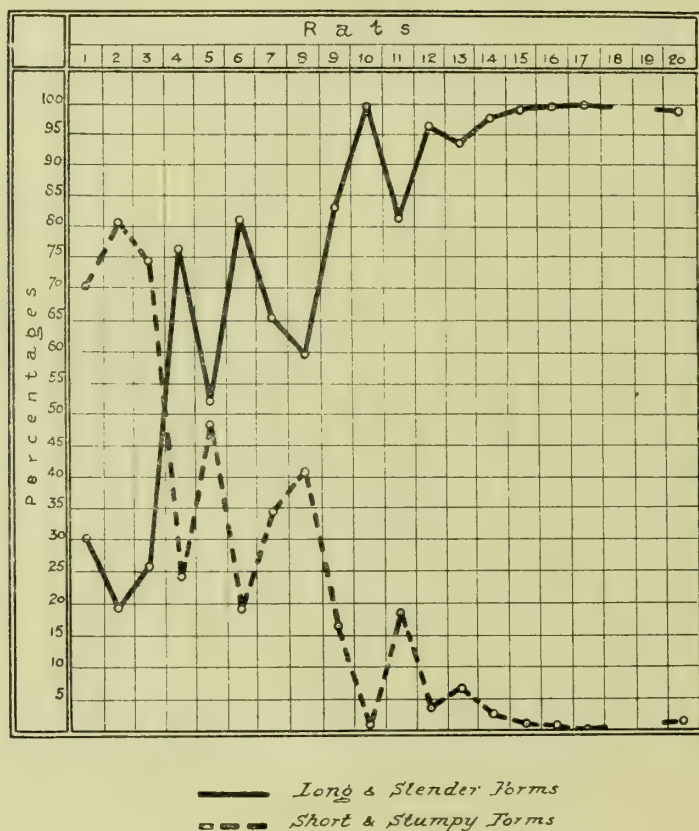
CHART 28.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, taken on 9 consecutive days from Rat 2471, after passage through rats for 2 years.



The curve is now practically monomorphic. The short and stumpy and the intermediate forms have almost disappeared, and only the long and slender survive. But, it may be objected, perhaps this curve from Rat 2471 is a mere accident, due to some peculiarity in the rat; it is possible that if another rat is inoculated from it the curve will be found to be as dimorphic in type as that of Rat 1218 on Chart 27. That this is not so will be seen by the following chart, which represents the gradual change in type which takes place in this trypanosome by passage through rats. The first Rat 67 was inoculated from the original dog; the second Rat 312 was inoculated from Rat 67; Rat 407 from Rat 312, and so on through a series of 20 rats. The unbroken line represents the percentage of the long and slender forms, the broken line the short and stumpy. For example, Rat 67 has 30 per cent. long and slender, and 70 per cent. short and stumpy. In Rat 670 the long and short forms are almost equally divided; Rat 786, 82 per cent. long and 18 per cent. short. The percentage of the long and slender gradually increases until, at the end of 17 passages, it reaches 100 per cent., so that from a dimorphic type with 70 per cent. short forms, the type gradually changes into a monomorphic type which has lost all the short forms and nothing but the long remain. This change was brought about in 15 months.

This seems to show how fallacious it is to reason from laboratory types of trypanosomes to the wild natural types, and probably accounts for the showers of new species which are constantly falling about our ears.

CHART 29. — Curves representing the Gradual Change of this Trypanosome from a Dimorphic Type to a Monomorphic.



Breadth.—The following table gives the breadth of Strain XXVI., from Dog 48.

Table LXII.—Measurements of the Breadth of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48.

Date.	Expt.	Animal.	No. of trypanosomes measured.	In Microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1912.	1218	Rat	500	2.90	5.00	1.35

Table LXIII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48.

Date.					Expt. No.	Animal.	Percentage among short and stumpy forms.
1912.							
April	22...	...	...	...	407	Rat	3
"	25...	...	...	...	407	"	2
"	29...	...	...	...	407	"	1
May	2...	...	...	...	407	"	4
"	6...	...	...	...	407	"	3
"	9...	...	...	...	407	"	8
"	13...	...	...	...	407	"	6
"	16...	...	...	...	407	"	8
"	20...	...	...	...	407	"	4
"	23...	...	...	...	407	"	23
"	27...	...	...	...	407	"	15
Average ...							7.0

Table LXIV.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, after passages through rats for seven months.

Date.					Expt. No.	Animal.	Percentage among short and stumpy forms.
1912.							
Sept.	5...	...	...	...	1218	Rat	5
"	6...	...	...	...	1218	"	3
"	7...	...	...	...	1218	"	4
"	8...	...	...	...	1218	"	0
"	9...	...	...	...	1218	"	0
"	10...	...	...	...	1218	"	0
"	11...	...	...	...	1218	"	0
"	12...	...	...	...	1218	"	0
"	13...	...	...	...	1218	"	1
"	14...	...	...	...	1218	"	0
Average ...							1.3

After passage through rats for two years the short and stumpy forms have nearly all disappeared and with them the posterior-nucleated forms. In Rat 407, on Table LXIII., there are a fair number of posterior-nucleated trypanosomes, on one day as many as 23 per cent., but this is exceptional.

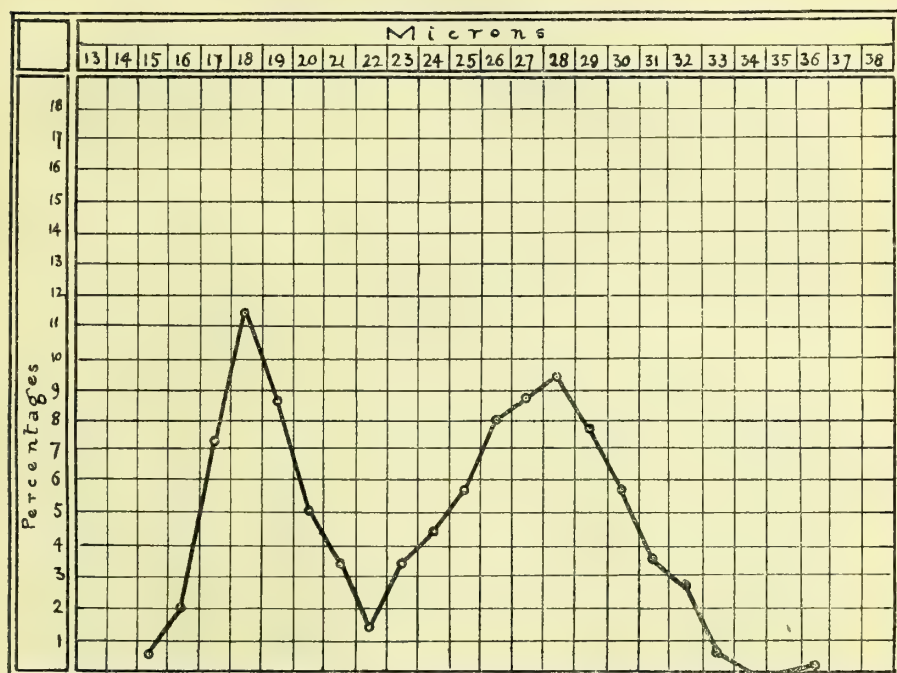
Morphology of the Naturally-Infected Dog Strain.

Strain XXVII., from Dog 690.

Table LXV.—Measurements of the Length of the Trypanosome of Naturally-Infected Dog, Strain XXVII., from Dog 690.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
July 26	911	Rat.	Osmic acid.	Giemsa.	25·3	32·0	18·0
" 26	911	"	"	"	23·6	30·0	18·0
" 26	911	"	"	"	25·0	31·0	19·0
" 27	911	"	"	"	24·9	32·0	15·0
" 27	911	"	"	"	25·9	32·0	18·0
" 27	911	"	"	"	27·0	32·0	17·0
" 30	911	"	"	"	24·2	31·0	18·0
" 30	911	"	"	"	25·4	30·0	18·0
" 30	911	"	"	"	25·0	31·0	18·0
" 31	911	"	"	"	25·9	32·0	19·0
" 31	911	"	"	"	25·2	31·0	16·0
" 31	911	"	"	"	26·9	32·0	18·0
Aug. 1	911	"	"	"	23·4	32·0	16·0
" 1	911	"	"	"	23·9	33·0	17·0
" 1	911	"	"	"	24·2	36·0	17·0
" 2	911	"	"	"	21·7	27·0	17·0
" 2	911	"	"	"	21·0	30·0	16·0
" 2	911	"	"	"	23·4	31·0	16·0
" 3	911	"	"	"	21·5	31·0	16·0
" 3	911	"	"	"	21·6	31·0	16·0
" 3	911	"	"	"	21·7	29·0	16·0
" 5	911	"	"	"	21·9	32·0	17·0
" 5	911	"	"	"	23·2	29·0	17·0
" 5	911	"	"	"	22·1	30·0	17·0
" 6	911	"	"	"	24·2	32·0	17·0
					23·9	36·0	15·0

CHART 30.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally-Infected Dog, Strain XXVII., from Dog 690, taken on 9 consecutive days from Rat 911.



The curve of Strain XXVII., from Dog 690, is also eminently dimorphic, so much so that the presence of two species might be suspected—one with a maximum of 18 microns and the other with a maximum of 28 microns. If such were the case, it could be argued that in Chart 27, of Strain XXVI., the long species had driven out the short. But it will be shown later that this is probably not so: that the difference is merely due to dimorphism and not to the mixture of two species of trypanosomes.

Breadth.—The following table gives the breadth of Strain XXVII., from Dog 690.

Table LXVI.—Measurements of the Breadth of the Trypanosome of Naturally-Infected Dog, Strain XXVII., from Dog 690.

Date.	Expt. No.	Animal.	No. of trypanosomes measured.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1912	911	Rat.	500	2.79	4.75	1.25

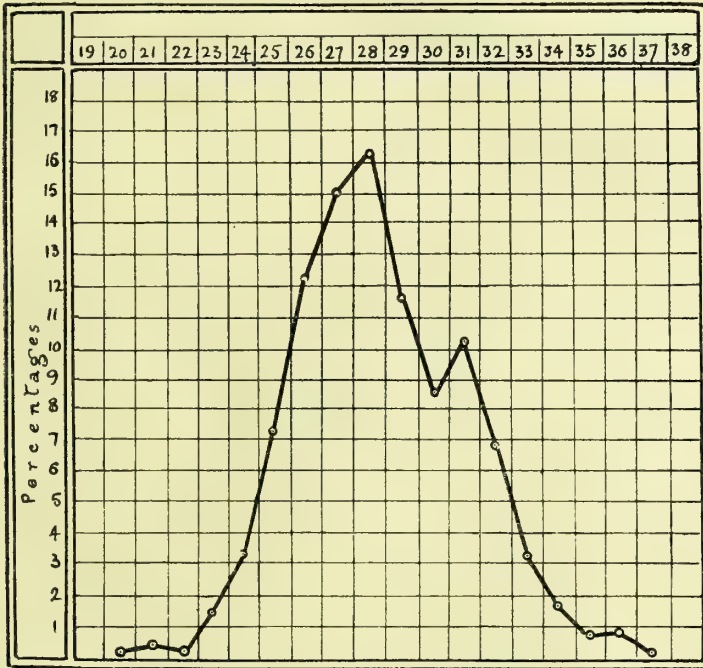
In regard to posterior-nuclear forms in this strain, there are practically none.

*Morphology of the Naturally-Infected Dog Strain.
Strain XXVIII., from Dog 2033.*

Table LXVII.—Measurements of the Length of the Trypanosome of Naturally-Infected Dog, Strain XXVIII., from Dog 2033.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.			
					Average length.	Maximum length.	Minimum length.	
1913.								
Apl.	10	2037	Rat	Osmic acid.	Giemsa.	29·6	34·0	24·0
"	10	2037	"	"	"	29·9	36·0	25·0
"	10	2037	"	"	"	30·2	36·0	24·0
"	11	2037	"	"	"	29·1	33·0	26·0
"	11	2037	"	"	"	29·5	33·0	25·0
"	11	2037	"	"	"	29·6	36·0	24·0
"	13	2037	"	"	"	29·7	35·0	25·0
"	13	2037	"	"	"	28·2	33·0	25·0
"	13	2037	"	"	"	29·6	33·0	25·0
"	14	2037	"	"	"	27·8	34·0	24·0
"	14	2037	"	"	"	29·7	37·0	25·0
"	14	2037	"	"	"	27·8	33·0	22·0
"	15	2037	"	"	"	28·1	32·0	25·0
"	15	2037	"	"	"	28·6	32·0	24·0
"	15	2037	"	"	"	27·5	31·0	25·0
"	16	2037	"	"	"	27·9	33·0	24·0
"	16	2037	"	"	"	28·1	33·0	24·0
"	16	2037	"	"	"	27·5	32·0	23·0
"	17	2037	"	"	"	26·8	31·0	21·0
"	17	2037	"	"	"	26·1	33·0	20·0
"	17	2037	"	"	"	26·1	29·0	23·0
"	19	2037	"	"	"	27·6	32·0	24·0
"	19	2037	"	"	"	28·5	36·0	25·0
"	19	2037	"	"	"	28·3	34·0	25·0
"	21	2037	"	"	"	27·9	31·0	22·0
						28·4	37·0	20·0

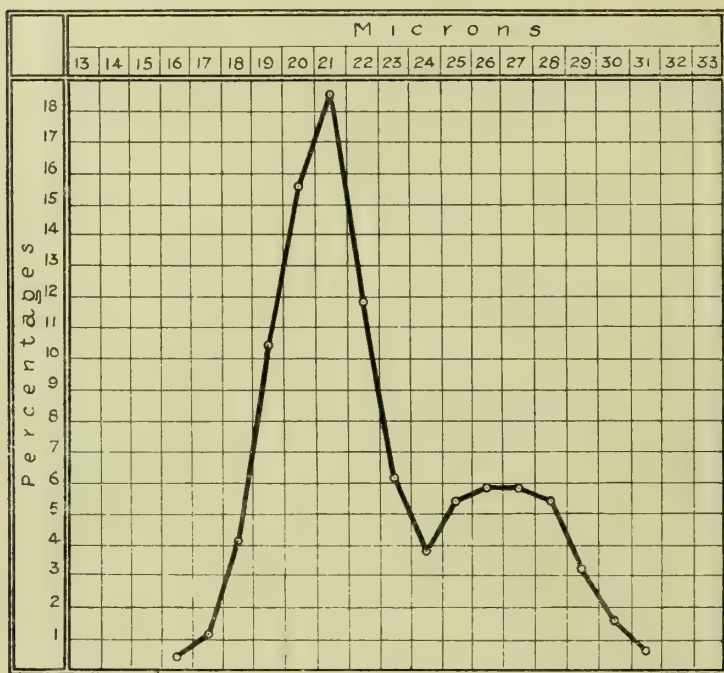
CHART 31.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally-Infected Dog, Strain XXVIII., from Dog 2033, taken on 9 consecutive days from Rat 2037.



This curve was taken from a rat which was inoculated directly from the Naturally-Infected Dog 2033, and had, therefore, passed through no series of rats. Yet the curve is the same as that shown in Chart 28, after two years' passage through rats.

It might be argued that this is really an infection with the larger of the two hypothetical species, the one having a maximum of 28 microns. It is to be regretted that this strain has died out, so that no further experimentation with it is possible. It would have been interesting to pass it through other animals, in order to learn if any reversion to the short and stumpy form would take place. But if it is not possible to do this with Strain XXVII., it is with Strain XXVI., which was seen to change from a dimorphic type to a practically monomorphic type after two years' passage through rats. When this almost-monomorphic rat strain, as shown in Chart 28 is inoculated into a dog, a reversion to the original dimorphic type is brought about, as will be seen from the following chart.

CHART 32.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally-Infected Dog, Strain XXVI., from Dog 48, taken from Dog 2498. This dog was inoculated from Rat 2471, which showed 95 per cent. long and slender forms.



In the blood of Rat 2471, Chart 28, the trypanosomes almost all belonged to the long and slender type. Now this is reversed and the majority are short and stumpy. This goes against the theory that two species are being dealt with in this strain. Passage through the rat favours the production of a long and slender monomorphic type of trypanosome, whereas passage from the rat to the dog at once changes this to a dimorphic type, in which the short and stumpy form the greater number.

Breadth.—The following table gives the breadth of the trypanosome of Strain XXVIII., from Dog 2033.

Table LXVIII.—Measurements of the Breadth of the Trypanosome of Naturally-Infected Dog, Strain XXVIII., from Dog 2033.

Date.	Expt. No.	Animal.	No. of trypanosomes measured.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1913	2037	Rat	500	2.61	4.00	1.50

In Strain XXVIII. there are no posterior-nucleated forms. This is not to be wondered at, as there are almost no short and stumpy forms, and it is only among them that posterior-nucleated trypanosomes are found.

Comparison of the Three Naturally-Infected Dog Strains with one another.

Table LXIX.—Measurements of the Length of the Trypanosome of the Naturally-Infected Dog, Strains XXVI., XXVII., and XXVIII.

Date.	Expt. No.	Strain.	Animal.	No. of trypanosomes measured.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	—	XXVI.	Rat	660	23·7	36·0	15·0
1912	911	XXVII.	„	500	23·9	36·0	15·0
1913	2037	XXVIII.	„	500	28·4	37·0	20·0

Table LXX.—Percentages of Short and Stumpy, Intermediate, and Long and Slender Forms in the Three Strains of the Naturally-Infected Dog.

Date.	Expt. No.	Strain.	Animal.	No. of trypanosomes measured.	Short and stumpy.	Intermediate.	Long and slender.
					15—21	2—24	25—37
1912	—	XXVI.	Rat	660	per cent. 47·1	per cent. 10·2	per cent. 42·7
1912	911	XXVII.	„	500	38·2	9·2	52·6
1913	2037	XXVIII.	„	500	0·6	4·8	94·6

Strains XXVI. and XXVII. are similar, but Strain XXVIII. differs so much from them that it would be useless to combine the three into one curve; the result would be misleading. In Strain XXVIII., as will be seen from Table LXX., almost all are long forms.

Table LXXI.—Measurements of the Breadth of the Trypanosome of the Naturally-Infected Dog, Strains XXVI., XXVII., and XXVIII.

Date.	Expt. No.	Strain.	Animal.	No. of trypanosomes measured.	In microns.		
					Average breadth.	Maximum breadth.	Minimum breadth.
1912	1218	XXVI.	Rat	500	2·90	5·00	1·25
1912	911	XXVII.	„	500	2·79	4·75	1·25
1913	2037	XXVIII.	„	500	2·61	4·00	1·50

Table LXXII.—Percentages of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Naturally-Infected Dog, Strains XXVI., XXVII. and XXVIII.

Date.	Expt. No.	Strain.	Animal	Percentage among short and stumpy forms.
1912	407	XXVI.	Rat	7·0
1912	911	XXVII.	„	0·1
1913	2037	XXVIII.	„	0·0

Comparison of the Naturally-Infected Dog Strain with the Human, Wild Game, and Wild Glossina Morsitans Strains of the Trypanosome causing Disease in Man in Nyasaland.

Table LXXIII.—Measurements of the Length of the Trypanosome of the Human, Wild Game, Wild *Glossina morsitans*, and Naturally-Infected Dog Strains.

Strain.	No. of trypanosomes measured.	Animal.	In microns.		
			Average length.	Maximum length.	Minimum length.
Human	5500	Rat	23·5	38·0	14·0
Wild Game	2500	„	22·6	35·0	15·0
Wild <i>G. morsitans</i> ...	2500	„	22·6	35·0	15·0
Naturally-Infected Dog	1660	„	25·5	37·0	15·0

Table LXXIV.—Measurements of the Breadth of the Trypanosome of the Human, Wild Game, Wild *Glossina morsitans*, and Naturally-Infected Dog Strains.

Strain.	No. of trypanosomes measured.	Animal.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
Human	1500	Rat	2·6	5·00	1·25
Wild Game	1500	„	3·2	5·75	1·50
Wild <i>G. morsitans</i> ...	1500	„	2·9	5·25	1·25
Naturally-Infected Dog	1500	„	2·8	5·00	1·25

Table LXXV.—Percentages of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human, Wild Game, Wild *Glossina morsitans*, and Naturally-Infected Dog Strains.

Date.	Strain.	Animal.	Percentage among short and stumpy forms.
1912	Human	Rat	17·8
1912	Wild Game	„	26·2
1912	Wild <i>G. morsitans</i>	„	12·5
1912	Naturally-Infected Dog	„	2·4

Comparison of the Morphology of the Naturally-Infected Dog Strain with the other Strains of the Trypanosome causing Disease in Man in Nyasaland.

At the outset it may be stated that it is impossible to separate the Naturally-Infected Dog Strain from the other strains by microscopical examination. As far as can be made out, it is identical in shape, size, and position of nucleus and micronucleus, contents of cell, and disposal of the undulating membrane.

Three plates are given at the end of this paper to illustrate the morphology of this strain, and, if they are compared with the plates given of the other strains,* this statement will be borne out.

On the other hand, there are very few posterior-nuclear forms—although, in one instance, they ran up to 23 per cent.—and, as a rule, the thick, blunt-ended type is not so common in this strain as in the others. But for all practical purposes it must be concluded that the Naturally-Infected Dog Strain is so similar in appearance to the others, that it would be impossible to separate it by morphology alone.

How this aberrant strain arose in these three chronically-infected dogs it is impossible to say. If it had been found anywhere else, in man, game, or fly, the position would have been simplified. But in none of them did anything like the Naturally-Infected Dog Strain appear. It was thought that perhaps the long sojourn in the blood of the dog had modified and weakened this strain, and attempts were made to prove this, but without success. All the dogs inoculated with the ordinary strains died in a few weeks, and inoculations from those which lingered longest showed no signs of weakening or change of any kind.

Conclusions.

1. The Naturally-Infected Dog Strain differs slightly from the other strains of the Trypanosome causing Disease in Man in Nyasaland, in that there are fewer of the posterior-nucleated, blunt-ended forms which are sometimes so much in evidence in the ordinary strains.

* "Proc." Roy. Soc., B, vol. 87, 1913, p. 135. *Ibid*, B, vol. 87, 1914, p. 510 ("Description of a Strain of *Trypanosoma brucei* from Zululand"). Also Reports of the Sleeping Sickness Commission, No. XV., p. 110, and above, p. 88.

2. Taking into consideration the fact that this strain was only found in three chronically-infected dogs, it is concluded that it is an aberrant strain of the widely-spread species, *Trypanosome brucei* vel *rhodesiense*, the Trypanosome causing Disease in Man in Nyasaland.

DESCRIPTION OF PLATES.

Trypanosome of Naturally-Infected Dog.

Plate 4.—Short and Stumpy, Non-flagellated Forms.

Plate 5.—Intermediate Forms.

Plate 6.—Long and Slender Forms.
× 2000.

XXII.—SUSCEPTIBILITY OF ANIMALS TO THE TRYPANOSOME—*T. BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE HUMAN STRAIN. STRAINS I. TO V.

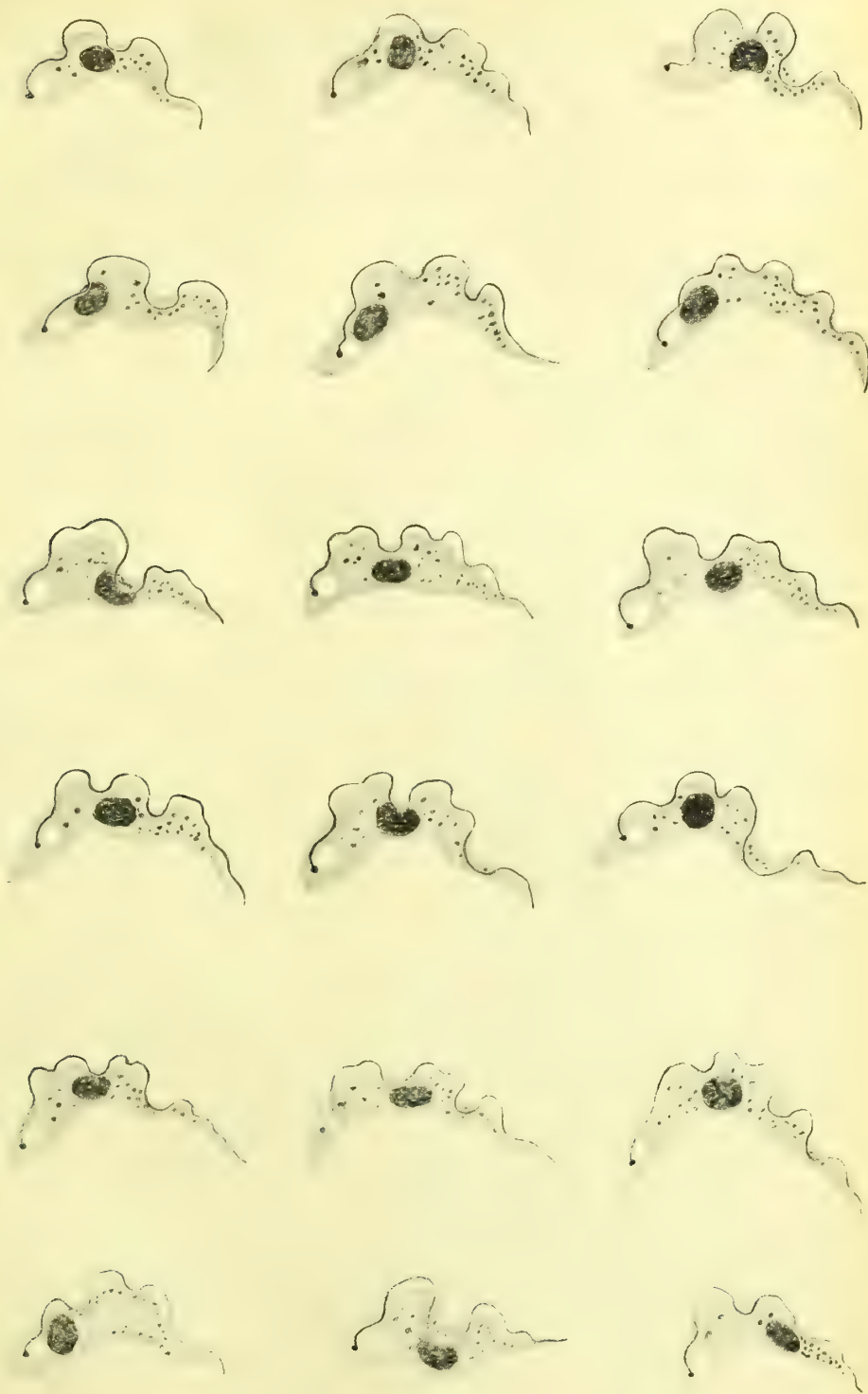
This paper has already been published in the "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 35, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 111.

The Human Strains I. to V. were all inoculated into animals and the results compared. It was concluded that:—

1. The trypanosome causing disease in man in Nyasaland is fatal to goats, sheep, dogs, and the smaller laboratory animals, killing them, without exception, in a few weeks. It is less virulent to cattle, many of which evidently escape.

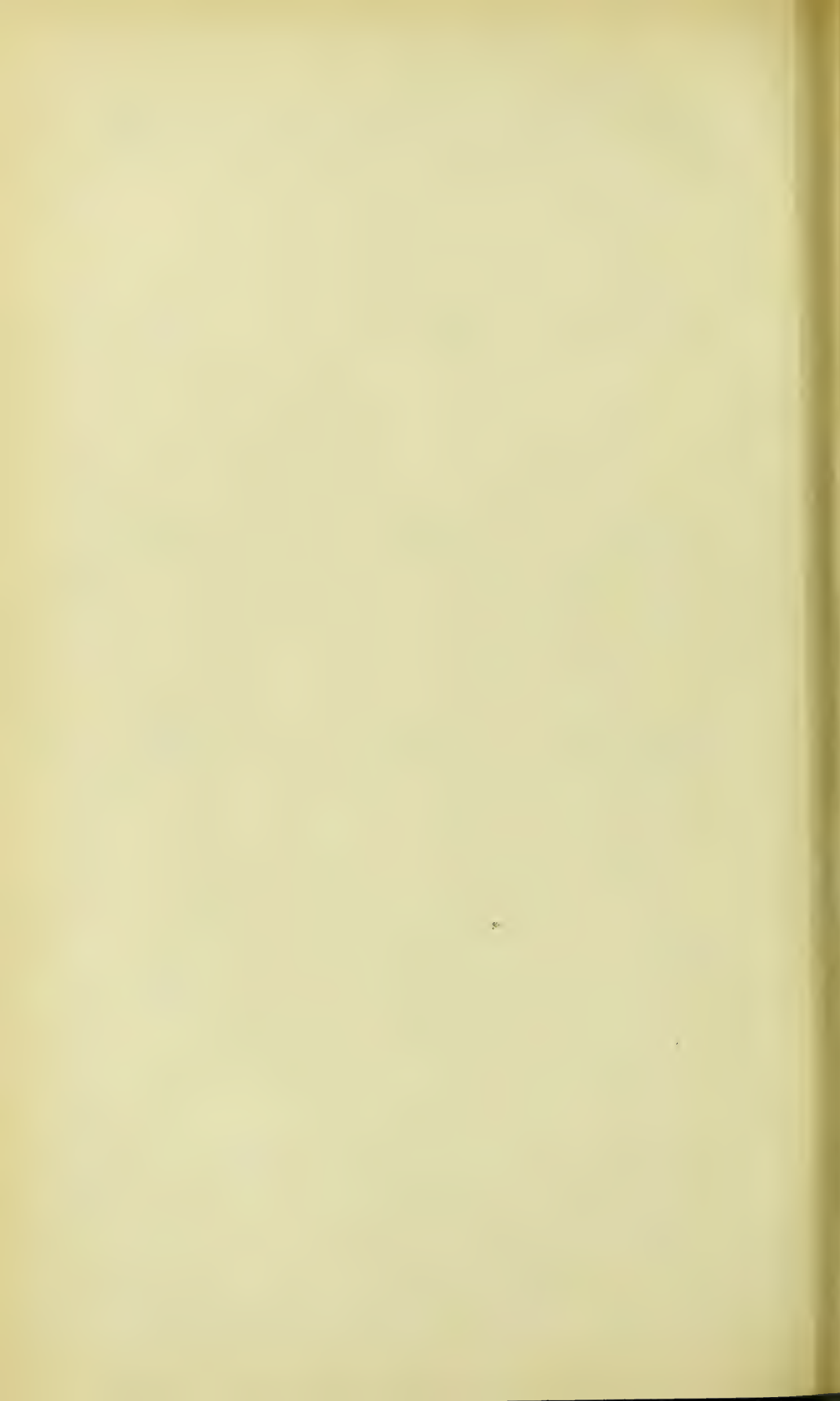
2. No difference in virulence can be made out in these five Human strains.

3. It is not satisfactorily proved yet to what species this trypanosome belongs, but the Commission at present leans to the opinion that it is *T. brucei* (Plimmer and Bradford).



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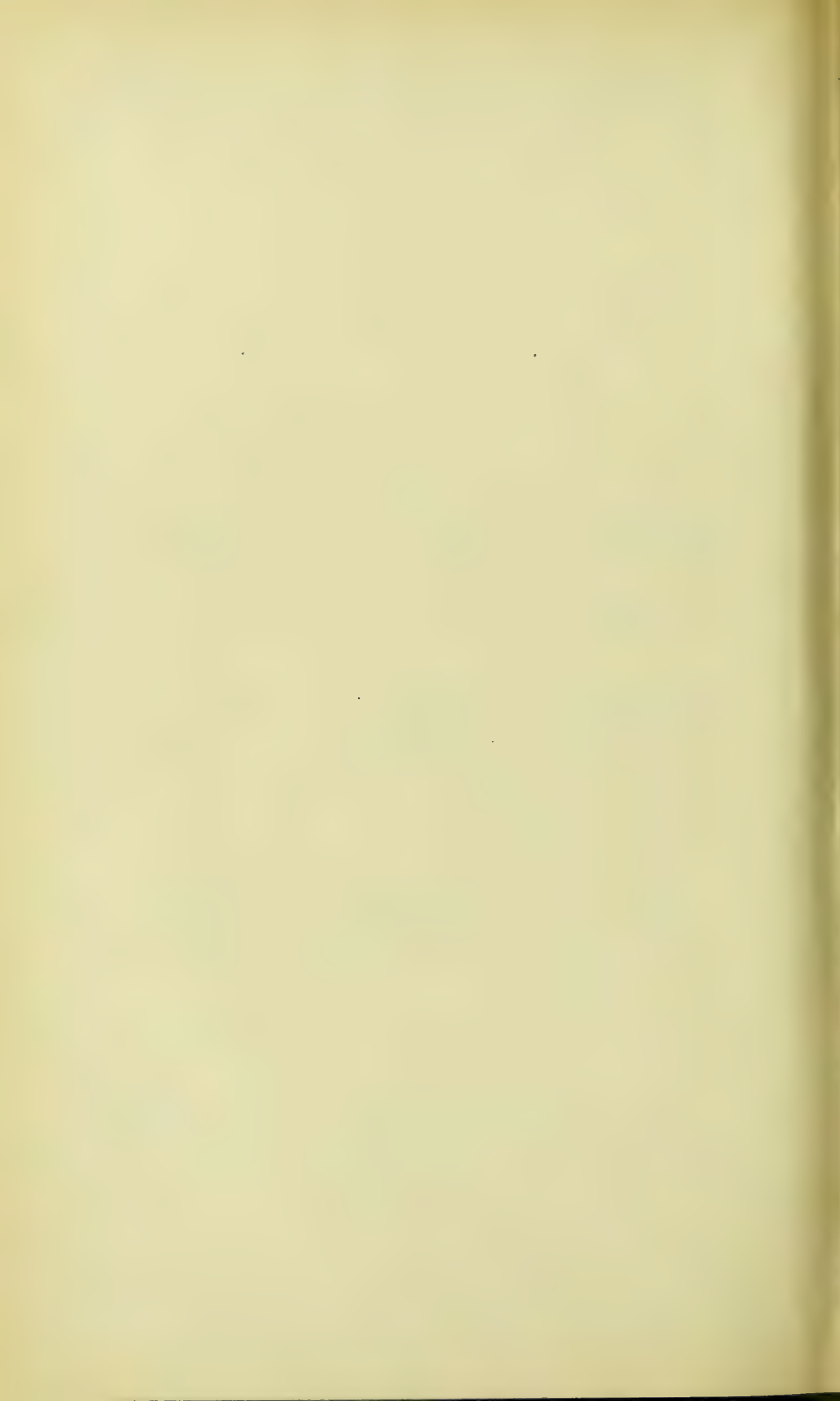
SHORT AND STUMPY FORMS.

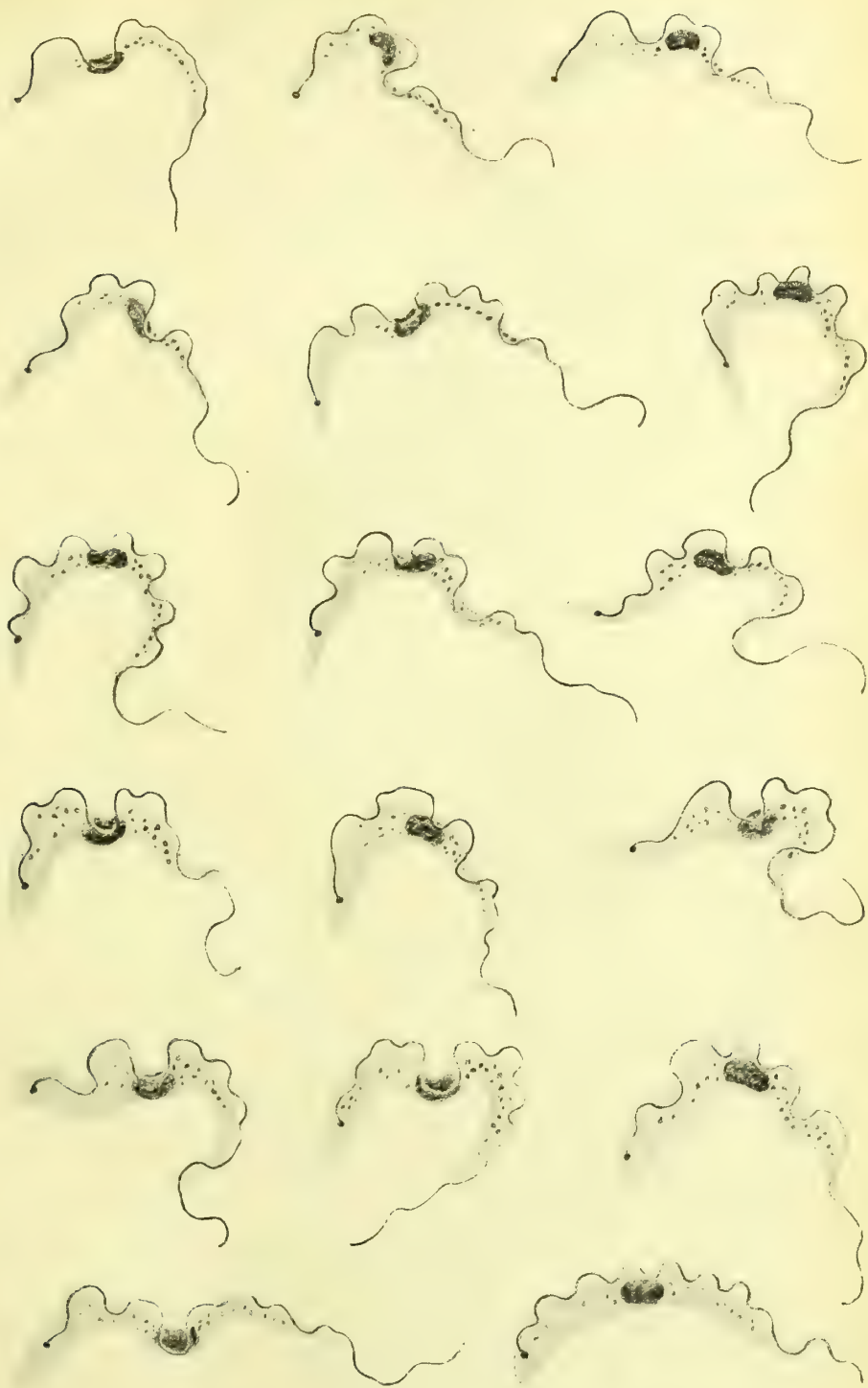




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INTERMEDIATE.





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LONG AND SLENDER FORMS.

XXIII.—SUSCEPTIBILITY OF ANIMALS TO THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE WILD GAME STRAIN.*

In a previous paper† the morphology of this strain of trypanosome was described, and it was concluded that it is identical with the trypanosome causing disease in man in Nyasaland, the *Trypanosoma rhodesiense* of Stephens and Fantham, the *Trypanosoma brucei* of this Commission.

Table LXXVI.

Date.	No. of Expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.‡	Remarks.
Goat.					
1912.					
July 1	718	Hartebeeste 779 ...	7	67	Died of wild game strain.
„ 23	796	„ 957 ...	13	60	„ „ „
„ 27	975	„ 1000 ...	9	27	„ „ „
Aug. 21	1126	„ 1142 ...	5	25	„ „ „
„ 24	1121	Eland 1202 ...	16	82	Also showed <i>T. pecorum</i> .
„ 24	1127	Waterbuck 1180 ...	9	49	Died of wild game strain.
„ 28	1218	„ 1210 ...	19	49	Also showed <i>T. caprae</i>
		Average ...	8.6	45.6	
Monkey.					
Jun. 30	785	Reedbuck 783 ...	8	17	Died of wild game strain.
July 1	776	Hartebeeste 779 ...	7	30	„ „ „
„ 8	864	Oribi 863 ...	7	59	„ „ „
„ 23	961	Hartebeeste 957 ...	13	21	„ „ „
„ 27	1001	„ 1000 ...	9	10	„ „ „
Aug. 24	1181	Waterbuck 1180 ...	9	40	„ „ „
„ 28	1211	„ 1210 ...	8	79	„ „ „
Sept. 13	1348	Reedbuck 1347 ...	10	19	„ „ „
„ 23	1436	„ 1435 ...	10	66	„ „ „
		Average ...	9.0	37.9	

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 205.

† "Proceedings" of the Royal Society, B, vol. 86, 1913, p. 394, and Reports of the Sleeping Sickness Commission, No. XV., p. 48.

‡ Duration includes the days of incubation; it dates from the day of infection.

Table LXXVI. (continued).

Date.	No. of expt.	Source of virus.			Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dog.							
Jun. 30	784	Reedbuck	783	...	8	11	Died of wild game strain.
July 1	733	Hartebeeste	779	...	7	58	" " "
" 10	846	Monkey	785	...	5	48	" " "
" 10	848	Dog	733	...	8	43	" " "
" 23	892	Hartebeeste	957	...	6	31	" " "
" 26	991	Monkey	864	...	6	48	" " "
" 27	1002	Hartebeeste	1000	...	9	59	" " "
Aug. 21	1144	"	1142	...	1	28	" " "
" 24	1182	Waterbuck	1180	...	9	40	" " "
" 28	1212	"	1210	...	8	26	" " "
Sept. 7	1266	"	1264	...	16	64	" " "
" 13	1349	Reedbuck	1347	...	10	38	" " "
" 23	1437	"	1435	...	10	42	" " "
Average					...	7.9	41.2
Rat.							
1912.							
July 10	847	Monkey	785	...	5	30	Died of wild game strain.
" 10	849	Dog	733	...	8	39	" " "
" 26	992	Monkey	864	...	6	21	" " "
Aug. 13	1070	Dog	1002	...	6	17	" " "
" 16	1022	Dog	892	...	3	30	" " "
Sept. 3	1220	Monkey	1181	...	3	54	" " "
Average					...	5.2	31.8

In making up the average incubation and duration, mixed infections are not included. It must be admitted that these averages are only approximate, as it is impossible to deal only with animals of the same age, weight, health, and powers of resistance. Dogs, for example, fall away very much during the rains, when biting flies and ticks are numerous.

Table LXXVII.—The Average Duration, in Days, of the Disease in Various Animals caused by the *Wild Game Strain* of the Trypanosome causing Disease in Man in Nyasaland.

	Goat.	Monkey.	Dog.	White rat.
Average duration in days ...	46.	38.	41.	32.
No. of animals employed ...	5	9	13	6

* Duration includes the days of incubation; it dates from the day of inoculation.

Table LXXVIII.—The Average Duration of Life, in Days, of Various Animals infected with the *Human Strain* of the Trypanosome causing Disease in Man in Nyasaland.

	Goat.	Monkey.	Dog.	White rat.
Average duration in days ...	42.	26.	24.	30.
No. of animals employed ...	29	20	25	21

Table LXXIX.—The Percentages of Recoveries in Various Animals infected with the *Wild Game Strain* of the Trypanosome causing Disease in Man in Nyasaland.

	Goat.	Monkey.	Dog.	White rat.
Percentages	0.	0.	0.	0.
No. of animals employed ...	5	9	13	6

Compare this with the following table:—

Table LXXX.—The Percentages of Recoveries in Various Animals infected with the *Human Strain* of the Trypanosome causing Disease in Man in Nyasaland.

	Goat.	Monkey.	Dog.	White rat.
Percentages	0.	0.	0.	0.
No. of animals employed ...	29	20	25	21

XXIV.—SUSCEPTIBILITY OF ANIMALS TO THE TRYPANOSOME—*T. BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE WILD *GLOSSINA MORSITANS* STRAIN.†

In a previous paper the morphology of this strain of trypanosome was described, and it was concluded that it is identical with the trypanosome causing Disease in Man in Nyasaland, the *T. brucei* vel *rhodesiense*.

Table LXXXI.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912.					
April 13	437	Dog 325 ...	9	-	Recovered.
" 14	438	" 325 ...	9		"
Goat.					
Jan. 21	35	Wild flies ...	19	35	Died of wild fly strain
Feb. 1	117	Monkey 20...	11	50	Also showed <i>T. simiae</i>
" 13	202	Wild flies ...	6	24	Died of wild fly strain
" 17	201	Dog 116 ...	12	27	" "
" 17	207	" 116 ...	9	40	" "
April 13	421	" 325 ...	19	130	" "
" 13	423	" 325 ...	9	82	" "
" 13	424	" 325 ...	9	99	" "
May 15	416	Wild flies ...	5	19	Also showed <i>T. simiae</i> and <i>T. pecorum</i>
June 12	637	Rat 543 ...	29	82	Died of wild fly strain
" 12	638	" 543 ...	12	46	" "
" 12	639	" 543 ...	12		Still alive after 224 days
" 27	716	Wild flies ...	10	60	Died of wild fly strain
Oct. 31	1538	" ...	8	24	Also showed <i>T. pecorum</i> and <i>T. caprae</i>
Nov. 23	1626	" ...	7	56	Died of wild fly strain
" 27	1638	" ...	15	64	Also showed <i>T. pecorum</i>
Dec. 5	1667	" ...	11	80	Also showed <i>T. pecorum</i> and <i>T. caprae</i>
" 9	1676	" ...	4	40	Also showed <i>T. caprae</i>
" 13	1685	" ...	7	38	Also showed <i>T. pecorum</i>
1913.					
April 16	2084	Rat 2020 ...	5	41	Died of wild fly strain
" 16	2085	" 2020 ...	19	32	" "
" 16	2086	" 2020 ...	8	32	" "
" 16	2087	" 2020 ...	8	39	" "
" 16	2088	" 2020 ...	8	44	" "
Average ...			11.8	54.3	

† Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 208.

* Duration includes the days of incubation; it dates from the day of inoculation.

Table LXXXI. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Pig.					
1912.					
Nov. 25	1636	Wild flies	14	98	Also showed <i>T. simiae</i> and <i>T. pecorum</i>
1913.					
Jan. 21	1781	"	6	280	Also showed <i>T. pecorum</i>
April 12	2075	"	11	24	Also showed <i>T. simiae</i>
May 14	2169	"	8	25	" "
Monkey.					
1912.					
Feb. 26	287	Dog 157	7	28	Died of wild fly strain
" 27	286	" 211	6	52	Also showed <i>T. simiae</i>
Mar. 13	332	Goat 117	8	31	Died of wild fly strain
April 13	406	Dog 325	9	71	" "
" 27	492	" 436	5	63	" "
May 8	523	Wild flies	4	5	Also showed <i>T. simiae</i>
" 31	601	"	6	31	Also showed <i>T. pecorum</i>
June 7	625	"	9	83	Died of wild fly strain
" 25	739	"	5	45	" "
July 24	970	Rat 658	8	—	Still alive after 162 days
Sept. 27	1459	Wild flies	9	13	Died of wild fly strain
Oct. 29	1536	"	10	12	" "
1913.					
Jan. 13	1757	"	6	32	Died of wild fly strain
May 14	2151	Rat 2082	5	30	" "
" 14	2152	" 2082	5	41	" "
" 14	2153	" 2082	5	22	" "
" 14	2154	" 2082	5	28	" "
" 14	2155	" 2082	5	33	" "
		Average	6.9	38.7	
Dog.					
1912.					
Feb. 1	116	Monkey 20... ..	8	23	Died of wild fly strain
" 16	211	Wild flies	6	—	Killed Mar. 2
" 17	157	Dog 116	9	11	Died of wild fly strain
" 17	243	" 116	5	23	" "
" 17	244	" 116	5	23	" "
Mar. 9	325	Monkey 286	9	41	" "
April 12	436	Wild flies	7	36	" "
" 13	440	Dog 325	5	29	" "
" 13	441	" 325	9	60	" "
" 27	493	" 436	5	60	" "
May 10	525	Wild flies	3	8	" "
" 13	542	"	6	42	" "
" 17	549	Monkey 523	10	51	Also showed <i>T. pecorum</i>
" 17	551	Wild flies	8	25	" "
" 29	595	"	3	18	" "

* Duration includes the days of incubation; it dates from the day of inoculation.

Table LXXXI. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dog—continued.					
1912.					
May 31	602	Wild flies ...	5	—	Still alive after 175 days
June 8	626	„ ...	8	53	Died of wild fly strain
„ 26	729	„ ...	5	26	„ „
July 24	971	Rat 658 ...	8	32	„ „
Oct. 30	1537	Wild flies ...	5	30	„ „
Nov. 22	1625	„ ...	4	30	„ „
„ 26	1637	„ ...	12	38	„ „
Dec. 7	1675	„ ...	6	25	„ „
„ 12	1684	„ ...	4	19	„ „
1913.					
Jan. 22	1782	Wild flies ...	9	21	Died of wild fly strain
May 14	2146	Rat 2082 ...	5	17	„ „
„ 14	2147	„ 2082 ...	8	17	„ „
„ 14	2148	„ 2082 ...	8	17	„ „
„ 14	2149	„ 2082 ...	5	11	„ „
„ 14	2150	„ 2082 ...	5	24	„ „
		Average ...	6.4	28.6	
Rabbit.					
1912.					
Apl. 13	439	Dog 325 ...	—	—	Never showed trypanosomes.
Dec. 14	1543	Pig 1636 ...	19	13	Died of wild fly strain
„ 14	1544	„ 1636 ...	19	39	„ „
„ 14	1545	„ 1636 ...	33	90	„ „
		Average ...	23.7	47.3	
Guinea-pig.					
Feb. 17	239	Dog 116 ...	16	80	Died of wild fly strain
„ 17	240	„ 116 ...	16	72	„ „
April 13	442	„ 325 ...	—	—	Never showed trypanosomes.
May 14	544	Monkey 492 ...	—	—	„ „
June 14	676	Dog 549 ...	—	—	„ „
„ 14	677	„ 549 ...	—	—	„ „
„ 14	678	„ 542 ...	13	116	Died of wild fly strain
„ 14	679	„ 542 ...	20	104	„ „
„ 14	680	„ 551 ...	13	39	Also showed <i>T. pecorum</i> .
„ 14	681	„ 551 ...	20	42	„ „
„ 14	682	„ 595 ...	—	—	Never showed trypanosomes.
„ 14	683	„ 595 ...	—	—	„ „

* Duration includes the days of incubation; it dates from the day of inoculation.

Table LXXXI. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Guinea-pig—continued.					
1913.					
Jan. 4	1731	Wild flies ...	15	100	Died of wild fly strain
Mar. 28	2034	Guinea-pig 1731 ...	10	53	" "
" 28	2035	" 1731 ...	10	89	" "
April 16	2077	Rat 2020 ...	15	61	" "
" 16	2078	" 2020 ...	12	61	" "
" 16	2079	" 2020 ...	8	72	" "
		Average ...	13.5	80.8	
Rat.					
1912.					
Feb. 17	241	Dog 116 ...	5	17	Died of wild fly strain
" 17	242	" 116 ...	5	15	" "
April 13	443	" 325 ...	9	24	" "
" 27	494	" 436 ...	12	14	" "
May 7	519	" 440 ...	6	12	" "
" 14	543	Monkey 492 ...	6	29	" "
" 17	550	Rat 519 ...	6	12	" "
June 11	655	Dog 549 ...	5	36	" "
" 11	656	" 542 ...	6	19	" "
" 11	657	" 551 ...	13	31	Also showed <i>T. pectorum</i>
" 11	658	" 595 ...	8	43	Died of wild fly strain
" 12	660	" 602 ...	5	70	Also showed <i>T. pectorum</i>
Dec. 3	1664	Monkey 970 ...	13	40	Died of wild fly strain
1913.					
Jan. 13	1755	Rat 1664 ...	7	71	" "
Mar. 25	2020	" 1755 ...	13	22	" "
" 25	2021	" 1755 ...	13	22	" "
April 16	2080	" 2020 ...	1	27	" "
" 16	2081	" 2020 ...	5	31	" "
" 16	2082	" 2020 ...	5	28	" "
" 16	2083	" 2020 ...	5	27	" "
May 15	2166	" 2082 ...	8	11	" "
		Average ...	7.3	26.3	

* Duration includes the days of incubation; it dates from the day of inoculation.

Table LXXXII.—The Average Duration, in Days, of the Disease in Various Animals caused by the Wild *Glossina morsitans* Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days	Rec.	54	38	29	47	81	26
Number of animals employed	2	16	14	25	3	10	19

Compare this with the following table:—

Table LXXXIII.—The Average Duration of Life, in Days, of Various Animals Infected with the *Human Strain* of the Trypanosome causing Disease in Man in Nyasaland.

	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days	134	42	26	34	28	67	30
Number of animals employed	1	29	20	25	7	15	21

Table LXXXIV.—The Percentages of Recoveries in Various Animals Infected with the Wild *Glossina morsitans* Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Percentages	100	6	7	4	0	0	0
Number of animals employed	2	17	15	26	3	10	19

Compare this with the following table:—

Table LXXXV.—The Percentages of Recoveries in Various Animals Infected with the *Human Strain* of the Trypanosome causing Disease in Man in Nyasaland.

	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Percentages	80	0	0	0	0	0	0
Number of animals employed	5	29	20	25	7	15	21

Comparison of the Wild Game and Wild Glossina Morsitans Strains with the Human Strain of the Trypanosome causing Disease in Man in Nyasaland.

Table LXXXVI.—The Average Duration, in Days, of the Wild Game, Wild *Glossina morsitans* and Human Strains of the Trypanosome causing Disease in Man in Nyasaland, in regard to their Virulence towards Various Animals.

Strain.	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Human	134	42	26	34	28	67	30
Wild Game	—	46	38	41	—	—	32
Wild <i>G. morsitans</i> ...	Rec.	54	38	29	47	81	26

Table LXXXVII.—The Percentages of Recoveries in Various Animals of the Wild Game, Wild *Glossina morsitans* and Human Strains of the Trypanosome causing Disease in Man in Nyasaland.

Strain.	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Human	80	0	0	0	0	0	0
Wild Game	—	0	0	0	—	—	0
Wild <i>G. morsitans</i> ...	100	6	7	4	0	0	0

Conclusions.

1. The pathogenic action on various animals of the *Human Strain*, the *Wild Game Strain*, and the *Wild Glossina morsitans Strain* is so much alike, that it may be concluded that they all three belong to the same species of trypanosome.

2. This species is *Trypanosoma brucei* vel *rhodesiense*, the Trypanosome causing Disease in Man in Nyasaland.

XXV.—SUSCEPTIBILITY OF ANIMALS TO THE TRYPANOSOME—*T. BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE LIWONDE STRAIN.*

In a previous paper† the morphology of this strain of trypanosome was described and it was concluded that it is identical with the trypanosome causing Disease in Man in Nyasaland, the *Trypanosoma brucei* vel *rhodesiense*.

The following tables give the incubation and duration of the disease in goats, monkeys, dogs, guinea-pigs, and white rats, which were sub-inoculated with Strains XXII., XXIII., and XXIV., from dogs infected in the Liwonde District.

Liwonde Strain XXII.

Table LXXXVIII.

Date.	No. of expt.	Source of virus.	Period of incuba- tion, in days.	Dura- tion of disease, in days.‡	Remarks.	
1913.		Goat.				
Aug. 12	2382	Monkey 2340	...	6	53	Died of strain XXII
„ 12	2383	„ 2340	...	6	44	„ „
		Monkey.				
Aug. 7	2339	Dog 2322	...	4	27	Died of strain XXII
„ 7	2339	„ 2322	...	4	72	„ „
		Dog.				
July 16	2322	Wild flies	...	6	23	Died of strain XXII
Aug. 12	2380	Monkey 2340	...	6	46	„ „
„ 12	2381	„ 2340	...	6	45	„ „
		Guinea-pig.				
Aug. 12	2384	Monkey 2340	...	13	78	Died of strain XXII
„ 12	2385	„ 2340	...	23	114	„ „
		Rat.				
Aug. 12	2378	Monkey 2340	...	6	35	Died of strain XXII
„ 12	2379	„ 2340	...	6	44	„ „

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 108.

† "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 97; and this Report, p. 59.

‡ Duration includes the days of incubation; it dates from day of inoculation.

Liwonde Strain XXIII.

Table LXXXIX.

Date.	No. of expt.	Source of virus.	Period of incuba- tion, in days.	Duration of disease, in days.*	Remarks.	
Goat.						
1913. Aug. 12	2366	Monkey 2336	...	6	39	Died of strain XXIII
„ 12	2367	„ 2336	...	9	41	„ „
Monkey.						
Aug. 6	2335	Dog 2323	...	5	32	Died of strain XXIII
„ 6	2336	„ 2323	...	5	43	„ „
Dog.						
July 5	2353	Wild flies	...	3	—	Killed 28th July
„ 28	2323	Dog 2353	...	9	17	Died of strain XXIII
Aug. 12	2364	Monkey 2336	...	6	23	„ „
„ 12	2365	„ 2336	...	6	27	„ „
Guinea-pig.						
Aug. 12	2368	Monkey 2336	...	49	94	Died of strain XXIII
„ 12	2369	„ 2336	...	—	—	Never showed try pansomes
Rat.						
Aug. 12	2391	Monkey 2336	...	5	22	Died of strain XXIII
„ 12	2363	„ 2336	...	6	21	„ „

Liwonde Strain XXIV.

Table XC.

Date.	No. of expt.	Source of virus.	Period of incuba- tion, in days.	Dura- tion of disease, in days.*	Remarks.	
Goat.						
1913.						
Aug. 12	2374	Monkey 2338	...	6	10	Died of strain XXIV
„ 12	2375	„ 2338	...	9	12	„ „
Monkey.						
Aug. 6	2337	Dog 2321	...	5	32	Died of strain XXIV
„ 6	2338	„ 2321	...	5	38	„ „

* Duration includes the days of incubation; it dates from day of inoculation.

Table XC. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dog.					
July 8	2356	Wild flies ...	4	—	Killed 28th July
„ 28	2321	Dog 2356 ...	9	—	Died of strain XXIV
Aug. 12	2372	Monkey 2338 ...	6	30	„ „
„ 12	2373	„ 2338 ...	6	12	„ „
Guinea-pig.					
Aug. 12	2376	Monkey 2338 ...	20	39	Died of strain XXIV
„ 12	2377	„ 2338 ...	73	93	„ „
Rat.					
Aug. 12	2370	Monkey 2338 ...	6	20	Died of strain XXIV
„ 12	2371	„ 2338 ...	6	17	„ „

Disease set up in Various Animals by the Trypanosome of the Liwonde Strain.*

The infection set up in the various animals by the Liwonde Strain gave rise to symptoms and appearances during life, and pathological changes in the various organs after death, alike and similar in every way to those caused by the Human Strain, Wild Game Strain, and the Wild *Glossina morsitans* Strain of *Trypanosoma brucei vel rhodesiense* found in the Proclaimed Area.

Comparison of the Three Liwonde Strains in regard to their Virulence towards Various Animals.

Table XCI.—The Average Duration, in Days, of the Disease in Various Animals.

Strain.	Goat.	Monkey.	Dog.	Guinea-pig.	White rat.
XXII. ...	48	49	38	96	30
XXIII. ...	40	37	22	94	21
XXIV. ...	11	35	22	66	18

No recoveries took place among the experimental animals.

* Duration includes the days of incubation; it dates from day of inoculation.

Table XCII.—The Average Duration of Life, in Days, of Various Animals Infected with the Liwonde Strain.

	Goat.	Monkey.	Dog.	Guinea-pig.	White rat.
Average duration, in days	33	41	28	84	26
Number of animals employed	6	6	9	5	6

Comparison of the Liwonde Strain with the Human Strain.

Table XCIII.—The Average Duration of Life, in Days, of Various Animals Infected with the Human and the Liwonde Strains.

	Strain.	Goat and sheep.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days...	Human	42	26	34	28	67	30
Average duration, in days...	Liwonde	33	41	28	—	84	26

Conclusions.

1. The pathological action on various animals of the Liwonde Strain is so much like that of the Human Strain that it may be concluded that these two belong to the same species of trypanosome.

2. This species is *Trypanosoma brucei vel rhodesiense*, the trypanosome causing disease in man in Nyasaland.

XXVI.—SUSCEPTIBILITY OF ANIMALS TO THE TRYPANOSOME—*TRYPANOSOMA BRUCEI VEL RHODESIENSE*—CAUSING DISEASE IN MAN. THE ZULULAND 1913 STRAIN. STRAIN XXV.*

In a foregoing paper† the morphology of this trypanosome was described, and the conclusion arrived at that it is identical, as regards shape, size and general appearance, with the trypanosome causing disease in man in Nyasaland, the *Trypanosoma brucei vel rhodesiense*.

This paper describes the action on animals of the Zululand Strain, and it is compared in this regard with the Nyasaland Strain.

* Reprinted, with amendments, from the "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 511.

† "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 493; and this Report, p. 71.

Table XCIV.

Date.	No. of expt.	Source of virus.	Incubation, in days.	Duration, in days.*	Remarks.
Horse.					
1895. —	37	Dog 4	3	35	"Very old animal; typical Nagana."—Shilston.
Sept. 27	212	Natural infection...	?	30	Zululand, 1896, Bruce
" 29	235	" " ...	6	49	" " "
		Average ...	—	38·0	
Ox.					
1913. —	22	Dog 4	6	—	"Still alive after 90 days."—Shilston.
Feb. 18	1913	Rat 1839	—	—	Never showed trypanosomes.
" 18	1914	" 1839	37	—	Still alive after 316 days.
" 18	1915	" 1839	35	310	Died of <i>T. brucei</i> .
July 22	2304	Dog 2281	—	—	Never showed trypanosomes.
" 22	2305	" 2281	—	—	" "
" 22	2306	" 2281	—	—	" "
Sheep.					
—	1	Dog 4	12	—	"Still alive after 90 days."—Shilston.
Goat.					
Feb. 12	1887	Rats 1832 and 1838	—	—	Never showed trypanosomes.
" 12	1888	" 1832 and 1838	—	—	" "
" 12	1889	" 1832 and 1838	15	100	Died of <i>T. brucei</i> .
" 12	1891	" 1832 and 1838	—	—	Never showed trypanosomes.
Mar. 15	1890	Guinea-pigs 1840 and 1843	12	45	Died of <i>T. brucei</i> .
July 16	2290	Dog 2254	26	39	" "
" 16	2291	" 2254	36	116	" "
		Average ...	22·2	77·2	

* Duration includes the days of incubation; it dates from day of inoculation.

Table XCIV. (continued).

Date.	No. of expt.	Source of virus.	Incubation, in days.	Duration, in days.*	Remarks.
Monkey.					
1913.					
Feb. 3	1833	Rabbit 1830 ...	7	8	Died of <i>T. brucei</i> .
" 3	1834	" 1830 ...	7	15	" "
" 3	1835	" 1830 ...	7	14	" "
" 3	1836	" 1830 ...	7	49	" "
" 3	1837	" 1830 ...	7	16	" "
" 17	1970	Laboratory-bred flies	—	17	" "
July 16	2292	Dog 2254 ...	5	50	" "
" 16	2293	" 2254 ...	5	65	" "
Average ...			6.4	29.2	
Dog.					
Feb. 14	1904	Monkey 1835 ...	6	26	Died of <i>T. brucei</i> .
" 14	1905	" 1835 ...	6	17	" "
" 14	1906	" 1835 ...	10	18	" "
" 14	1907	" 1835 ...	6	23	" "
" 14	1908	" 1835 ...	6	21	" "
April 1	2047	Rat 2027 ...	6	15	" "
" 1	2048	" 2027 ...	6	20	" "
" 1	2049	" 2027 ...	6	14	" "
" 1	2050	" 2027 ...	6	17	" "
" 1	2051	" 2027 ...	6	20	" "
" 22	2104	" 2065 ...	6	17	" "
June 24	2240	Guinea-pig 2225 ...	7	12	" "
July 3	2254	Laboratory-bred flies	?	17	" "
" 9	2281	Guinea-pig 2225 ...	5	20	" "
" 16	2294	Dog 2254 ...	5	19	" "
" 16	2295	" 2254 ...	5	22	" "
Aug. 11	2361	Wild flies ...	?	16	" "
Average ...			6.1	18.5	
Rabbit.					
—	145	Dog 1 ...	—	33	Shilston.
—	146	" 1 ...	—	39	" "
—	150	" 2 ...	—	31	" "
—	157	Rat 4A ...	13	34	" "
—	158	Horse 37 ...	3	27	" "
—	165	Rabbit 158... ..	5	27	" "
Jan. 13	1897	Pretoria strain ...	?	36	Died of <i>T. brucei</i> .
" 17	1830	" " ...	?	35	" "
Average ...			7.0	32.7	

* Duration includes the days of incubation; it dates from day of inoculation.

Table XCIV. (continued).

Date.	No. of expt.	Source of virus.	Incubation, in days.	Duration, in days.*	Remarks.
Guinea-pig.					
1913.					
Feb. 3	1840	Rabbit 1830 ...	31	50	Died of <i>T. brucei</i> .
" 3	1841	" 1830 ...	35	50	" "
" 3	1842	" 1830 ...	—	—	Never showed trypanosomes.
" 3	1843	" 1830 ...	21	59	Died of <i>T. brucei</i> .
" 3	1844	" 1830 ...	14	37	" "
" 13	1895	Pretoria strain ...	—	—	Never showed trypanosomes
" 13	1896	" " ...	—	—	" "
Mar. 28	1842	Monkey 1970 ...	10	27	Reinjected; died of <i>T. brucei</i> .
" 28	1895	" 1970 ...	20	46	" "
" 28	1896	" 1970 ...	20	30	" "
May 13	2136	" 1970 ...	—	—	Never showed trypanosomes.
" 29	2136	" 1970 ...	4	15	Reinjected; died of <i>T. brucei</i> .
June 13	2225	" 1970 ...	3	34	Died of <i>T. brucei</i> .
July 16	2296	Dog 2254 ...	47	89	" "
		Average ...	20.5	43.7	
Rat.					
Feb. 2	1828	Rabbit 1830 ...	8	19	Died of <i>T. brucei</i> .
" 2	1829	" 1830 ...	6	58	" "
" 3	1832	" 1830 ...	7	46	" "
" 3	1838	" 1830 ...	10	13	" "
" 3	1839	" 1830 ...	7	22	" "
" 14	1902	" 1897 ...	17	31	" "
" 26	1966	Guinea-pig 1844 ...	8	23	" "
Mar. 15	1993	Rat 1832 ...	5	13	" "
" 15	1994	" 1832 ...	5	10	" "
" 15	1995	" 1832 ...	5	30	" "
" 19	2006	Monkey 1970 ...	5	24	" "
April 4	2065	" 1836 ...	7	30	" "
" 11	2073	Rat 2065 ...	4	17	" "
May 13	2135	Monkey 1970 ...	6	33	" "
" 13	2137	Goat 1889 ...	6	18	" "
" 13	2138	" 1889 ...	6	17	" "
" 29	2196	Rat 2135 ...	11	26	" "
July 16	2288	Dog 2254 ...	5	33	" "
" 16	2289	" 2254 ...	5	44	" "
Sept. 2	2406	" 2361 ...	6	24	" "
" 16	2412	Rat 2406 ...	6	34	" "
Oct. 20	2423	" 2412 ...	8	39	" "
Nov. 28	2442	" 2431 ...	4	16	" "
		Average ...	6.8	27.0	

* Duration includes the days of incubation; it dates from day of inoculation.

*Disease set up in Various Animals by T. brucei, Zululand
Strain XXV., 1913.*

Horse.—The Commission had no opportunity of studying this strain in the horse, but Mr. Shilston states that one horse inoculated by him at Pietermaritzburg died in 35 days with typical symptoms of Nagana.

Ox.—Six oxen were inoculated, but only two of these at any time showed trypanosomes in their blood. One of these died after 310 days, while the other is still alive at the end of a year. This animal has evidently recovered, as it appears sleek and healthy. The action of the Zululand strain is therefore the same as that of the trypanosome causing disease in man in Nyasaland: neither of them show any marked power of producing serious disease in cattle.

Goat.—Seven goats were inoculated with this strain. Four died, on an average, in 77.2 days (45 to 116). The remaining three proved refractory. No œdema of face or corneal opacity was noted in any of the goats. The Zululand strain seems to have less action on goats than the Nyasaland trypanosome, but the number of experiments is small. In the latter the duration of the disease was 41.8 days (19 to 72).

Sheep.—No experiments were made with these animals in Nyasaland as it was found impossible to procure them from the natives.

Monkey.—Eight monkeys died, on an average, in 29.2 days (8 to 65). The trypanosomes were always present in the blood, sometimes in enormous numbers. In no case was œdema of the face or corneal opacity noted. After death, enlargement of the spleen and liver, gelatinous infiltration at the base of the heart, and hæmorrhages in the epicardium were found.

Dog.—Seventeen dogs were inoculated. All died, on an average, in 18.5 days (12 to 26). In 8 dogs blindness caused by opacity of the cornea was a prominent symptom, and in two swellings of the limbs were observed.

Rabbit.—As only two rabbits were available at Kasu, six experiments reported by Mr. Shilston are added. Eight rabbits died, on an average, in 32.7 days (27 to 39). The course of the disease in the Kasu rabbits was the same as that described in a former paper* as being typical of Nagana.

Guinea-pig.—This animal is less affected by the disease than the rabbit. Ten were used; all took the disease and died, but four required to be inoculated more than once. They died, on an average, in 43.7 days (15 to 89). No prominent symptoms, such as are seen in the rabbit, occur in the guinea-pig.

Rat.—Twenty-three were inoculated and died, on an average, in 27 days (10 to 58), with their blood swarming with trypanosomes and their spleens enormously enlarged.

* "The Trypanosome causing Disease in Man in Nyasaland.—Susceptibility of Animals to the Human Strain," "Proceedings" of the Royal Society, B, vol. 87 (1913); and Reports of the Sleeping Sickness Commission, No. XV., p. 111.

Table XCV.—The Average Duration, in Days, of the Disease in Various Animals caused by *T. brucei*, Zululand Strain XXV., 1913.

	Horse.	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days	38	310	77	29	18	33	44	27
Number of animals employed	3	1	7	8	17	8	10	23

Compare this with the following table:—

Table XCVI.—The Average Duration of Life, in Days, of Various Animals Infected with the Human Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Horse.	Ox.	Goat and sheep.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days	0	134	42	26	34	28	67	30
Number of animals employed	0	1	29	20	25	7	15	21

Table XCVII.—The Percentages of Recoveries in Various Animals Infected with *T. brucei*, Zululand Strain XXV., 1913.

	Horse.	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Percentages ...	0	83	0	0	0	0	0	0
Number of animals employed	3	6	4	8	17	8	10	23

Compare this with the following table:—

Table XCVIII.—The Percentages of Recoveries in Various Animals Infected with the Trypanosome causing Disease in Man in Nyasaland.

	Horse.	Ox.	Goat and sheep.	Monkey.	Dog.	Rabbit.	Guinea- pig.	White rat.
Percentages ...	0	80	0	0	0	0	0	0
Number of ani- mals employed	0	5	29	20	25	7	15	21

Conclusion.

The pathogenic action of *T. brucei*, Zululand 1913 Strain XXV., on various animals is so similar, not only in regard to the symptoms during life but also in the *post-mortem* appearances and rate of mortality, to that of the trypanosome causing disease in man in Nyasaland, that it affords another proof that these two trypanosomes are identical.

XXVII.—SUSCEPTIBILITY OF ANIMALS TO THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE NATURALLY-INFECTED DOG STRAIN. STRAINS XXVI. TO XXVIII.*

In a previous paper† the morphology of the three strains of this trypanosome, from three naturally-infected dogs, was described, and the strains compared with each other and with the Human Strain.

This paper describes the action on various animals of the three strains, and tabulates a comparison with the Human Strain.

The first strain—XXVI.—was studied in a fairly large number of animals, but the second and third in few, as both were accidentally lost.

* Reprinted from the "Proceedings" of the Royal Society, vol. 88, 1914, p. 130.

† "Proceedings" of the Royal Society, vol. 88, 1914, p. 111; and p. 88 of this Report.

Table XCIX.—Strain XXVI., from Dog 48.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Cattle.					
1912. Mar. 6	314	From Dog 210 ...	—	—	Never showed trypanosomes
„ 6	315	„ 210 ...	—	—	„ „
April 13	314	From Rat 311 ...	12	—	Still „ alive after 335 days
„ 13	315	„ 311 ...	—	—	Never showed trypanosomes
Goat.					
Mar. 6	275	From Dog 210 ...	—	—	Never showed trypanosomes
„ 6	277	„ 210 ...	—	—	„ „
April 5	275	From Rat 312 ...	24	—	Accidentally killed
„ 5	277	„ 312 ...	—	—	Never showed trypanosomes
„ 20	427	„ 392 ...	10	—	Still alive after 277 days
„ 20	432	„ 392 ...	26	—	„ „
1913. Mar. 21	2008	„ 1991 ...	—	—	Never showed trypanosomes
„ 21	2009	„ 1991 ...	27	—	Still alive after 251 days
„ 21	2010	„ 1991 ...	—	—	Never showed trypanosomes
„ 21	2011	„ 1991 ...	17	—	Died of pneumonia
„ 21	2012	„ 1991 ...	—	—	Never showed trypanosomes
„ 21	2013	„ 1991 ...	—	—	„ „
„ 21	2014	„ 1991 ...	24	—	Still „ alive after 251 days
„ 21	2015	„ 1991 ...	17	—	„ „
„ 21	2016	„ 1991 ...	—	—	Never showed trypanosomes
„ 21	2017	„ 1991 ...	—	—	„ „
Sheep.					
1912. April 20	456	From Rat 392 ...	5	—	Still alive after 340 days
„ 20	457	„ 392 ...	10	64	Died of strain XXIV
Antelope.					
May 21	2059	From Rat 2024 ...	13	—	Still alive after 250 days

* Duration includes the days of incubation; it dates from the day of inoculation.

Table XCIX. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Monkey.					
1912.					
Mar. 6	318	From Dog 210 ...	—	—	Never showed trypanosomes
April 5	318	From Rat 312 ...	—	—	" "
" 20	50	From Dog 317 ...	—	—	" "
" 20	453	From Rat 392 ...	—	—	" "
Oct. 29	1533	" 1491 ...	—	—	" "
" 29	1534	" 1491 ...	6	—	Still alive after 148 days
Nov. 22	1629	From Monkey 1534	—	—	Never showed trypanosomes
" 22	1630	" 1534	10	—	Still alive after 124 days
1913.					
Jan. 22	1792	From Rat 1741 ...	5	—	Still alive after 186 days
" 22	1793	" 1741 ...	5	—	" "
" 22	1794	" 1741 ...	—	—	Never showed trypanosomes
" 22	1798	From Monkey 1630	—	—	" "
Feb. 28	1794	From Rat 1945 ...	—	—	" "
" 28	1798	" 1945 ...	—	—	" "
May 22	1794	From Monkey 2131	—	—	" "
" 22	1798	" 2131	—	—	" "
June 11	1794	" 2184	—	—	" "
" 11	1798	" 2184	—	—	" "
Dog.					
1912.					
Feb. 17	210	From Rat 67 ...	8	25	Died of strain XXVI
Mar. 6	317	From Dog 210 ...	—	—	Never showed trypanosomes
" 13	331	From Rat 67 ...	—	—	" "
April 6	317	" 312 ...	12	16	Died of strain XXVI
" 13	331	" 311 ...	12	46	" "
" 20	458	From Dog 317 ...	—	—	Never showed trypanosomes
" 20	459	" 317 ...	19	—	Still alive after 224 days
Sept. 6	1253	From Rat 1218 ...	6	—	Still alive after 120 days
Oct. 29	1525	" 1491 ...	13	30	Died of strain XXVI
" 29	1526	" 1491 ...	23	53	" "
" 29	1527	" 1491 ...	—	—	Never showed trypanosomes
" 29	1528	" 1491 ...	13	21	Died of strain XXVI
" 29	1529	" 1491 ...	13	47	" "
" 29	1530	" 1491 ...	16	—	Still alive after 148 days

* Duration includes the days of incubation; it dates from the day of inoculation.

Table XCIX. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dog—continued.					
1913.					
Jan. 22	1795	From Rat 1741 ...	5	29	Died of strain XXVI
„ 22	1796	„ 1741 ...	8	35	„ „
„ 22	1797	„ 1741 ...	22	—	Still alive after 131 days
April 14	2054	Laboratory-bred flies	7	30	Died of strain XXVI
May 29	2197	From Dog 2054 ...	11	16	„ „
„ 29	2198	„ 2054 ...	4	13	„ „
Dec. 30	2483	From Rat 2471 ...	14	34	„ „
1914.					
Jan. 26	2498	From Dog 2483 ...	4	11	„ „
		Average ...	11·6	29·0	
Rabbit.					
1912.					
Mar. 30	389	From Rat 67 ...	9	256	Died of strain XXVI
„ 30	390	„ 67 ...	17	109	„ „
		Average ...	13·0	182·5	
Guinea-pig.					
Mar. 6	313	From Dog 210 ...	—	—	Never showed trypanosomes.
April 5	313	From Rat 312 ...	—	—	„ „
„ 20	460	„ 392 ...	—	—	„ „
„ 20	461	„ 392 ...	—	—	„ „
„ 29	1531	„ 1491 ...	—	—	„ „
„ 29	1532	„ 1491 ...	—	—	„ „
Nov. 13	1531	„ 1492 ...	—	—	„ „
„ 13	1532	„ 1492 ...	—	—	„ „
1913.					
Jan. 17	1775	„ 1734 ...	—	—	„ „
June 17	2228	„ 2215 ...	—	—	„ „
„ 17	2229	„ 2215 ...	6	—	Still alive after 246 days.
July 22	2307	„ 2285 ...	—	—	Never showed trypanosomes.
„ 22	2308	„ 2285 ...	—	—	„ „
„ 31	2307	„ 2285 ...	—	—	„ „
„ 31	2308	„ 2285 ...	—	—	„ „

* Duration includes the days of incubation; it dates from the day of inoculation.

Table XCIX. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Rat.					
1913.					
Mar. 26	311	From Rat 67 ...	13	18	Died of strain XXVI.
" 26	312	" 67 ...	9	60	" "
" 30	391	" 67 ...	9	21	" "
" 30	392	" 67 ...	9	21	" "
April 5	407	" 312 ...	10	54	" "
" 20	462	" 407 ...	10	25	" "
May 25	585	" 407 ...	5	28	" "
June 14	670	" 585 ...	6	27	" "
July 2	786	" 670 ...	6	45	" "
Aug. 2	1020	" 786 ...	6	30	" "
" 30	1218	" 1020 ...	3	94	" "
Oct. 19	1492	" 1218 ...	9	54	" "
Dec. 12	1687	" 1492 ...	4	16	" "
" 28	1719	" 1687 ...	9	32	" "
1913					
Jan. 3	1734	" 1570 ...	9	36	" "
" 3	1735	" 1570 ...	6	39	" "
" 7	1741	" 1719 ...	4	15	" "
" 9	1749	" 1719 ...	4	18	" "
" 27	1814	" 1749 ...	7	16	" "
Feb. 10	1855	" 1814 ...	3	20	" "
" 22	1945	" 1855 ...	5	17	" "
Mar. 7	1985	" 1945 ...	6	20	" "
" 25	2022	" 1985 ...	6	16	" "
" 25	2023	" 1985 ...	6	18	" "
" 25	2024	" 1985 ...	6	19	" "
April 10	2070	" 2023 ...	4	12	" "
" 22	2105	" 2070 ...	6	20	" "
May 7	2124	" 2105 ...	3	8	" "
" 13	2134	From Dog 2054 ...	2	12	" "
" 25	2183	From Rat 2133 ...	3	7	" "
" 15	2168	" 2124 ...	7	17	" "
" 13	2133	From Dog 2054 ...	6	10	" "
June 10	2214	" 2197 ...	3	10	" "
" 18	2230	From Rat 2214 ...	8	10	" "
Aug. 13	2389	" 2280 ...	8	48	" "
Sept. 6	2409	" 2389 ...	5	49	" "
" 16	2413	" 2409 ...	6	70	" "
Oct. 23	2425	" 2413 ...	5	64	" "
Nov. 12	2432	" 2425 ...	6	38	" "
Dec. 20	2471	" 2432 ...	6	22	" "
1914					
Jan. 16	2484	" 2471 ...	7	16	" "
Average ...			6.2	28.6	

* Duration includes the days of incubation; it dates from the day of inoculation.

Table C.—Strain XXVII., from Dog 690.

Date.	No. of expt.	Source of virus.	Period of incubation in days.	Duration of disease, in days.*	Remarks.
Monkey.					
1913 Sept. 13	1314	From Dog 690 ...	—	—	Never showed trypanosomes.
„ 13	1315	„ 690 ...	—	—	„ „
Dog.					
1912 June 17	690	Naturally-infected	?	—	Recovered.
Guinea-pig.					
1913 Sept. 13	1316	From Dog 690 ...	—	—	Never showed trypanosomes.
„ 13	1317	„ 690 ...	—	—	„ „
Rat.					
1912 July 18	911	From Dog 690 ...	7	30	Died of strain XXVII

Table CI.—Strain XXVIII., from Dog 2033.

Date.	No. of expt.	Source of virus.	Period of incubation in days.	Duration of disease, in days.*	Remarks.
Goat.					
1913 May 21	2174	From Rat 2089 ...	12	71	Cause of death uncertain.
„ 21	2175	„ 2089 ...	—	—	Never showed trypanosomes.
„ 21	2176	„ 2089 ...	—	—	„ „
„ 21	2177	„ 2089 ...	—	—	„ „
„ 21	2178	„ 2089 ...	12	—	Died of pneumonia.
Monkey.					
May 14	2161	From Rat 2091 ...	—	—	Never showed trypanosomes.
„ 14	2162	„ 2091 ...	8	—	Recovered.
„ 14	2163	„ 2091 ...	—	—	Never showed trypanosomes.
„ 14	2164	„ 2091 ...	—	—	„ „
„ 14	2165	„ 2091 ...	12	—	Recovered.
June 14	2061	From Dog 2157 ...	—	—	Never showed trypanosomes.
„ 14	2164	„ 2157 ...	—	—	„ „

* Duration includes the days of incubation; it dates from the day of inoculation.

Table CI. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days,	Duration of disease, in days.*	Remarks.
Dog.					
Mar. 28	2033	Rat-infected ...	?	?	Died 1st April.
May 14	2156	From Rat 2091 ...	15	40	Died of strain XXVIII.
" 14	2157	" 2091 ...	8	33	" "
" 14	2158	" 2091 ...	8	26	" "
" 14	2159	" 2091 ...	15	93	" "
" 14	2160	" 2091 ...	15	102	" "
Average ...			12·2	58·8	
Guinea-pig.					
Mar. 28	2039	From Dog 2033 ...	—	—	Never showed trypanosomes.
" 28	2040	" 2033 ...	—	—	" "
May 21	2180	From Rat 2089 ...	22	—	Recovered.
" 21	2181	" 2089 ...	—	—	Never showed trypanosomes.
Rat.					
Mar. 28	2037	From Dog 2033 ...	13	73	Died of Strain XXVIII.
April 16	2089	From Rat 2037 ...	5	15	" "
" 16	2090	" 2037 ...	5	87	" "
" 16	2091	" 2037 ...	5	28	" "
May 14	2167	" 2091 ...	8	57	" "
Oct. 23	2426	From Guinea-pig 2180 ...	11	19	" "
Average ...			7·8	46·5	

Disease set up in Various Animals by the Trypanosome causing Disease in Man in Nyasaland. The Naturally-Infected Dog Strain.

Ox.—This trypanosome does not appear to be virulent to the ox. Four experiments were made. The trypanosomes appeared in the blood of one of the oxen, and it was returned as "Recovered" after being under observation for 335 days. The parasites were only seen on three occasions in this ox, and then only in scanty numbers.

Goat.—The trypanosome also has little effect on goats. Twenty-one were inoculated. Of these, 12 proved refractory; five showed the trypanosomes in their blood on one or two occasions in very scanty numbers, and were returned as "Recovered" after being

* Duration includes the days of incubation; it dates from the day of inoculation.

under observation for nearly a year; four died, one from the result of an accident, two from pneumonia, and the remaining one only once showed the trypanosomes, and, as no *post-mortem* examination was made, it is impossible to say what was the cause of death. It may, therefore, be said that not a single goat of the 21 died of the disease.

Sheep.—Two sheep were inoculated. One recovered; the other died after 64 days, probably of the disease.

Monkey.—This trypanosome has little or no effect on monkeys. Twenty-seven were used as experimental animals. Twenty-one proved refractory; the remaining six were returned as "Recovered," after being under observation for several months.

Dog.—This strain has become, after several passages, virulent to dogs. Twenty-eight were used for experiment. Nineteen died, on an average, in 36.8 days (11 to 102); four never showed trypanosomes in their blood; and five recovered. The *post-mortem* appearances are the same as those found in Nagana: enlargement of the spleen, gelatinous œdema about the vessels at the base of heart, petchiæ of mucous membranes; and corneal opacity.

Rabbit.—Only two were inoculated. Both died, one after 109 days, the other after 256 days. Both showed corneal opacity and presented the same symptoms as those described in Nagana rabbits, but in a much milder degree.

Guinea-pig.—The Guinea-pig, like the monkey, is almost refractory to this strain. Twenty-one animals were inoculated. Nineteen of these proved refractory, and the remaining two only showed trypanosomes on one occasion and appear to have recovered. Rats inoculated with their blood remain unaffected.

White rat.—This strain is virulent to rats. Forty-eight were inoculated and all died, on an average, in 30.8 days (7 to 94), with enormous enlargement of spleen, and the blood swarming with trypanosomes.

Comparison of the Three Strains of the Trypanosome of the "Naturally-Infected Dog Strain" in regard to their Virulence towards Various Animals.

Table CII.—The Average Duration, in Days, of the Disease in Various Animals of the Three Strains. The Letter R means that the Animals are Refractory.

Strain.	Ox.	Goat.	Sheep.	Mon-key.	Dog.	Rabbit.	Guinea-pig.	White rat.
XXVI.	R	R	64	R	29	182	R	29
XXVII.	—	—	—	R	—	—	R	30
XXVIII.	—	R	—	R	59	—	R	46

Table CIII.—The Average Duration, in Days, of the Disease in Various Animals of the Three Strains combined. The Letter R stands for Refractory.

	Ox.	Goat.	Sheep.	Mon-key.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days	R	R	64	R	37	182	R	31
No. of animals employed	4	21	2	27	19	2	21	48

Table CIV.—The Percentages of Recoveries in Various Animals Infected with the Naturally-Infected Dog Strain. Three Strains combined.

	Ox.	Goat.	Sheep.	Mon-key.	Dog.	Rabbit.	Guinea-pig.	White rat.
Percentages ...	100	100	50	100	21	0	100	0
No. of animals employed	1	7	2	6	24	2	2	48

From Table CIV. it will be seen that the Naturally-Infected Dog Strain is not fatal to oxen, goats, monkeys, or guinea-pigs, whereas it killed 79 per cent. of the dogs and 100 per cent. of the white rats.

Comparison of the Trypanosome of the Naturally-Infected Dog Strain with the Trypanosome causing Disease in Man in Nyasaland (Trypanosoma brucei vel rhodesiense), Human Strain.

Table CV.—The Average Duration of Life, in Days, of Various Animals Infected with the Naturally-Infected Dog Strain and the Human Strain. The Letter R stands for Refractory.

Strain.	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Naturally infected dog	R	R	R	37	182	R	31
Human ...	134	42	26	34	28	67	30

It is curious that this strain, although evidently harmless to oxen, goats, monkeys, and guinea-pigs, is quite as virulent as the Human Strain to dogs and rats.

Table CVI.—The Percentages of Recoveries in Various Animals Infected with the Naturally-Infected Dog Strain and the Human Strain.

Strain.	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Naturally infected dog	100	100	100	21	0	100	0
Human	80	0	0	0	0	0	0

This shows the great difference in regard to action on animals which exists between the Naturally-Infected Dog Strain and the Human Strain,* and, if similar tables referring to other strains—for example, the Zululand 1913 Strain†—be compared, the same difference is found. It might be said that this alone is sufficient to make it rank as another species, and, as already mentioned, if this strain had been found among the wild game and wild *Glossina morsitans* in Nyasaland, this would have been justified. It was, however, only found in three chronically-infected dogs, and so it is thought best, with our present knowledge, to include it among the strains of *Trypanosoma brucei vel rhodesiense*.

If in the future it should be decided to give it specific rank, the name *Trypanosoma anceps* is suggested. This name seems appropriate on account of the uncertainty which exists as to the classification of this trypanosome.

Conclusions.

1. The Naturally-Infected Dog Strain is fatal to dogs, rabbits, and white rats, but oxen, goats, monkeys, and guinea-pigs appear to be refractory.

2. The Commission is of opinion that this is an aberrant or exceptional variety of strain of the Trypanosome causing Disease in Man in Nyasaland—*Trypanosoma brucei vel rhodesiense*.

* "Proc." Roy. Soc., B, vol. 87, 1913, p. 44; and Reports of the Sleeping Sickness Committee, No. XV., p. 111.

† "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 511; and this Report, p. 119.

XXVIII.—DEVELOPMENT IN *GLOSSINA MORSITANS* OF
THE TRYPANOSOME—*T. BRUCEI* VEL *RHODESIENSE*
—CAUSING DISEASE IN MAN IN NYASALAND. THE
HUMAN STRAIN.*

In previous papers† the morphology of this trypanosome and the susceptibility of various animals to its pathogenic action have been described. In this, is given an account of its development in *Glossina morsitans*.

In Uganda the study of the development of *Trypanosoma gambiense* in *G. palpalis* was much assisted by the circumstance that large numbers of laboratory-bred tsetse flies were available. This was due to the fact that the pupæ of *G. palpalis* could be collected on the lake-shore in practically unlimited numbers. It is quite otherwise with *G. morsitans*. It has been found impossible to find the pupæ of this species in any numbers, so that all laboratory-bred *G. morsitans* have had to be hatched out of pupæ obtained from captive flies, a slow and laborious process. The flies are caught some 20 to 30 miles from the laboratory and brought up to Kasu camp by a native on a bicycle. This kills a large number of the flies. Moreover, the climatic conditions at the camp are not always favourable for breeding and hatching out. This was remedied to some extent by establishing a breeding station down in the low-country, but, as this had to be left in the charge of natives, the results were not always very satisfactory.

The study of the development of this trypanosome in *G. morsitans* has, therefore, been rendered difficult by the small number of laboratory-bred tsetse flies which could be obtained. Over and above that, flies bred from captive flies are not so strong and healthy as those hatched out from wild pupæ.

An attempt was made to use wild flies by feeding batches of about 20 on healthy animals and picking out those cages which did not give rise to infection. But this is at best a roundabout and clumsy method, as it can never be certain, although every care is taken, that only clean flies are being dealt with.

The Development of the Trypanosome causing Disease in Man in Nyasaland in G. morsitans.

Eleven experiments were carried out with laboratory-bred flies. Three were positive and eight negative.

Five experiments were also carried out with wild flies, as no laboratory-bred flies were available. All were positive.

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 516.

† "Proc." Roy. Soc., B, vol. 85 (1912), p. 423, and B, vols. 86 and 87 (1913), pp. 285 and 35. Also Reports of the Sleeping Sickness Commission, No. XV., pp. 1, 31, and 111.

Tables CVII. and CVIII. show these 16 experiments: the number of flies used; the number of infected flies found on dissection; and the number of days which elapsed before the flies became infective. As each fly died it was dissected and the result noted. As will be seen from Table CVII., several infected flies were found in the negative experiments. This probably means that the flies were only infected, not infective. The number of days before a fly becomes infective is arrived at by deducting seven days from the number of days which elapsed between the first infected feed of the flies and

Table CVII.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1912						
May 22	563	18	—	0		
June 13	668	22	—	1		
July 15	879	32	—	7		
„ 29	1003	28	+	2	31	84° F. (29° C.)
Aug. 17	1072	27	—	3		
Oct. 23	1494	22	—	3		
Nov. 6	1560	19	—	0		
Dec. 13	1686	24	—	2		
„ 23	1710	30	—	0		
„ 30	1723	35	+	3	14	84° F. (29° C.)
1933						
Aug. 31	2405	30	+	4	23	84° F. (29° C.)

the appearance of trypanosomes in the blood of the experimental animal. Seven days is put down as the average number of days between the infection of the animal and the appearance of the trypanosomes in its blood—the incubation period. It is probably a day or two shorter.

The number of flies used in each experiment was small, due to the difficulty of obtaining laboratory-bred flies. They were kept during the experiment in the incubator at a temperature of 84° F. (29° C.).

In Experiment 1723 the number of days which elapsed before the flies became infective is only 14. This number is obtained, as mentioned above, by deducting seven days for the incubation period, but this may have been a day or two less. The flies were kept at an evenly-warm temperature, which would tend materially to shorten the period of development. Still, 14 days seems a short time to elapse between the first feed on the infected animal and the appearance of an infective fly in the cage.

Two hundred and eighty-seven laboratory-bred flies were used and 25 infected flies were found—8.7 per cent.

Table CVIII.—Wild Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1912						
Dec. 11	1680	80	+	8	18	84° F. (29° C.)
„ 13	1688	40	+	6	3	84° F. (29° C.)
„ 18	1705	45	+	7	1	84° F. (29° C.)
1913						
Jan. 9	1748	70	+	1	25	84° F. (29° C.)
„ 14	1729	20	+	1	30	84° F. (29° C.)

Experiments 1688 and 1705 are evidently cases of infection by naturally-infected wild flies which had escaped detection. They are included in the table as they both show invasion of the salivary glands and so help to throw light on the mode of development of this trypanosome in *G. morsitans*. The other three pass through an interval of 18, 25, and 30 days before the cages became infective. These are probably cases where there was no naturally-infected fly in the cage, and these periods, therefore, represent the usual length of time required for the cycle of development of this trypanosome to take place in *G. morsitans*. The wild flies were also kept in the incubator at a temperature of 84° F.

Two hundred and fifty-five flies were used and 23 infected flies were found—9 per cent.

Details of the Eight Positive Experiments.

The following table gives the details of the eight positive experiments:—

Table CIX.

Expt.	Day of expt.	Procedure.	Remarks.
1003	1—2 3 4—4	Flies fed on infected dog Starved Fed on clean monkey 1023	Trypanosomes appeared in blood of monkey 1023 on the 38th day.
1723	1—41 5 6—22	Flies fed on infected dog Starved Fed on clean monkey 1733	Trypanosomes appeared in blood of monkey 1733 on the 21st day.
2405	1—6 7 8—32	Flies fed on infected monkey Starved Fed on clean monkey 2410	Trypanosomes appeared in blood of monkey 2410 on the 30th day.

Table CIX. (continued).

Expt.	Day of expt.	Procedure.	Remarks.
1680	1—2 3 4—22	Flies fed on infected dog Starved Fed on clean dog 1708	Trypanosomes appeared in blood of dog 1708 on the 25th day.
1688	1—2 3 4—12	Flies fed on infected monkey Starved Fed on clean monkey 1699	Trypanosomes appeared in blood of monkey 1699 on the 10th day.
1705	1—2 3 4—9	Flies fed on infected monkey Starved Fed on clean monkey 1707	Trypanosomes appeared in blood of monkey 1707 on the 8th day.
1748	1—2 3 4—30	Flies fed on infected monkey Starved Fed on clean monkey 1845	Trypanosomes appeared in blood of monkey 1845 on the 32nd day.
1729	1—2 3 4—38	Flies fed on infected dog Starved Fed on clean dog 1767	Trypanosomes appeared in blood of dog 1767 on the 37th day.

Omitting Experiments 1688 and 1705, it would appear from the remaining six experiments that an average period of 24 days is required to complete the cycle of development of the trypanosome causing Disease in Man in Nyasaland in *G. morsitans*, the flies being kept at a temperature of 84° F.

Details of the Eight Negative Experiments.

The following table shows the method of procedure in carrying out the eight negative experiments:—

Table CX.

Expt.	Day of expt.	Procedure.	Remarks.
563	1—3 4 5—52	Flies fed on infected monkey Starved Fed on clean monkey 594	All flies negative on dissection.
668	1—2 3 4—63	Flies fed on infected dog Starved Fed on clean dog 699	One infected fly found on the 42nd day.
879	1—2 3 4—32 33—63	Flies fed on infected monkey Starved Fed on clean monkey 910 Fed on clean monkey 1073	Seven infected flies found.

Table CX. (continued).

Expt.	Day of expt.	Procedure.	Remarks.
1072	1—3 4 33—63	Flies fed on infected dog Starved Fed on clean dog 1148	Three infected flies found.
1494	1—3 4—5 6—44	Flies fed on infected monkey Starved Fed on clean monkey 1514	Three infected flies found.
1560	1—3 4 5—37	Flies fed on infected monkey Starved Fed on clean monkey 1581	All flies negative on dissection.
1686	1—4 5 6—43	Flies fed on infected monkey Starved Fed on clean monkey 1704	Two infected flies found.
1710	1 2 3—47	Flies fed on infected dog Starved Fed on clean monkey 1718	All flies negative on dissection.

Result of the Dissection of the Infected Flies.

All the flies dying during the progress of these experiments were dissected.

In the three positive experiments with the laboratory-bred flies nine infected flies were found. The following table gives the results of the dissection of these nine flies. The second column gives the number of days which elapsed between the fly's first infected feed and its death and dissection. In the third column the labial cavity and hypopharynx are included under "Proboscis." At the time these experiments were made, no attempt was made to distinguish between the two parts, as has been done lately in the case of *T. simia*.* When the proboscis is marked positive, as in Table CXII., it may be that the trypanosomes are contained in the labial cavity or the hypopharynx, or both.

In the development of *T. gambiense* in *G. palpalis* trypanosomes were never noted as occurring in the proboscis.† In this species they are noted on several occasions as occurring in this position, but only in the wild-fly experiments, not in the laboratory bred. It seems natural to expect that if the salivary glands are swarming

* "Proc." Roy. Soc., B., vol. 87, p. 59 (1913), and Reports of the Sleeping Sickness Commission, No. XV., p. 131.

† Proceedings of the Royal Society, B., vol. 82 (1910), p. 368, and B., vol. 83, 1911, p. 345; also Reports of the Sleeping Sickness Commission, No. XI., pp. 12 and 34.

with trypanosomes that some of them will sometimes appear in the hypopharynx, and, moreover, in the wild flies some of the infections of the proboscis are no doubt due to *T. pecorum*, *T. simia*, or *T. capra*, all of which develop in the proboscis.

Table CXI.—Laboratory-bred Flies. Result of the Dissection of the Infected Flies found in the Positive Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
1003	33	—			+		—
1003	39	—			+		?
1723	30	—	++	++	++	++	—
1723	30	—	++	++	++	++	—
1723	48	—	—	—	—	—	—
2405	32				+		—
2405	33	—	++	++	++	++	++
2405	33	—	—	+	+	+	—
2405	33	—	—	+	+	+	—

In Experiment 1003, two infected flies were found. The first had only a gut infection, and, unfortunately, it was found impossible to dissect out the salivary glands of the second. Neither had an infection of the proboscis.

In Experiment 1723, three infected flies were found. The first and second had the alimentary tract swarming with flagellates, but none in the salivary glands. The third was found, on dissection, to be free from trypanosomes throughout. This is curious, because this fly had been isolated in a glass tube as an infective fly, and had, when used alone on a rat and rabbit, infected both these animals. The fly remained alive in the tube for 13 days, and the only explanation that can be given is that in this case the trypanosomes disappeared absolutely from the fly some few days before its death. This was the first time this had been observed to take place, and it was thought to be a remarkable phenomenon and difficult to credit, until another example of the same kind was observed. It must, therefore, be held as probable that an infective fly, with presumably both salivary glands and alimentary tract swarming with trypanosomes, can lose all these flagellates and become non-infective.

In Experiment 2405, four infected flies were found. Three of these were infections limited to the gut. The fourth was a good example of a salivary-gland infection. The glands were swarming with trypanosomes, and a portion of one of them injected under the skin of Rat 2417 gave rise to infection.

Table CXII.—Wild Flies. Result of the Dissection of the Infected Flies found in the Positive Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Foregut.	Midgut.	Hindgut.	Salivary glands.
1680	5	—	—	—	+	—	—
1680	19	—	—	—	+	—	—
1680	32	+	++	++	++	++	++
1680	32	—	—	+	+	+	—
1680	33	—	++	++	++	++	—
1680	33	—	—	—	+	—	—
1680	33	—	—	++	++	++	—
1680	33	—	—	+	+	—	—
1688	10	—	—	—	+	—	—
1688	10	—	—	—	+	—	—
1688	11	—	—	—	+	—	—
1688	3	—	—	—	+	—	—
1688	15	—	++	++	++	++	++
1688	15	+	—	—	+	—	—
1705	8	+	+	+	+	—	—
1705	8	—	+	+	+	—	—
1705	10	—	—	—	+	—	—
1705	11	—	—	+	—	—	—
1705	12	+	+	+	+	+	++
1705	26	—	—	++	++	++	++
1705	33	—	+	++	++	++	++
1748	31	—	—	++	++	++	++
1729	48	+	+	+	+	+	++

In Experiment 1680, eight flies were found to be infected. In seven, the flagellates were confined to the alimentary tract. The eighth had a well-marked invasion of the salivary glands. In this case, trypanosomes were also seen in the proboscis, but whether in the labial cavity or the hypopharynx is not specified.

In Experiment 1688, six flies were found to contain trypanosomes in the alimentary canal. In one of these there was also infection of the salivary glands, which were crowded with trypanosomes. This fly must have been naturally infected when caught, as sufficient time had not elapsed since the infected feed to allow of time for development to take place. The flagellates contained in the salivary glands injected into Rat 1721 gave rise to infection.

In Experiment 1705, seven infected flies were found. Three of these had the salivary glands invaded. One of these, the fifth, must also have been a naturally-infected wild fly.

In Experiment 1748, only one infected fly was found. It had a copious infection of the salivary glands, a portion of which injected into Rat 1852 gave a positive result.

In the last Experiment, 1729, there was also only one infected fly found. The salivary glands were swarming with trypanosomes.

The next table gives the result of the dissection of the infected flies found in the experiments which remained negative.

In the negative Experiments 563, 1560, and 1710, none of the flies were found to be infected with trypanosomes in any part (see Table CVII.). These experiments are, therefore, omitted from this table.

Table CXIII.—Laboratory-bred Flies. Result of the Dissection of the Infected Flies found in the Negative Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Foregut.	Midgut.	Hindgut.	Proctodæum.	Salivary glands.
668	42	—		+	+	+		—
879	7	—	—	+	+	+		—
879	8	—		+	++			—
879	9	—		+	+	—		—
879	11	—			+			—
879	24	—	+	+	+	+	—	—
879	28	—	++	++	++	++		—
879	40	—	++	++	++	+		—
1072	7	—	—	+	+	+		—
1072	10	—		+	+	+		—
1072	38	—	++	++	++	++		++
1494	7	—	++	—	—	—		—
1494	17	—		+	+	—		—
1494	31	—		+	+			—
1686	8	—	—	++	++	+		—
1686	26		—	+	+	+		—

From these negative experiments it will be seen that only in one fly did an infection of salivary glands occur. Why this fly did not infect the animal it fed on is impossible to say.

The Methods used in the Examination of the Flies.

The flies were dissected as described in a previous paper.* As each fly in a cage died it was dissected, and the result, as regards the presence of trypanosomes in the alimentary tract and salivary glands, recorded. Fixed and stained preparations were then made from the various parts and numerous drawings of the various types of trypanosomes encountered were made. The method described in a previous paper† of isolating infective flies and inducing them to salivate on clean cover-glasses was also made use of. This is a useful, simple and practical method, as it demonstrates clearly the type of trypanosome thrown out from the tip of the proboscis when the fly feeds.

The Trypanosomes found in the Alimentary Tract.

In this species of trypanosome, the developmental changes which take place in the intestine of *G. morsitans* are similar to those already described as occurring in the development of *T. gambiense* in *G. palpalis*.‡ The latter development has also been worked out very fully and completely by others.§ It is, therefore, unnecessary

* "Proc." Roy. Soc., B, vol. 83, p. 513 (1911), and Reports of the Sleeping Sickness Commission, No. XI., p. 34.

† "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 63, and Reports of the Sleeping Sickness Commission. No. XV., p. 137.

‡ "Proceedings" of the Royal Society, B, vol. 83 (1911), p. 515; also Reports of the Sleeping Sickness Commission No. XI., p. 34.

§ Muriel Robertson, M.A., "Phil. Trans. of the Royal Society," B, vol. 203 (1913).

here to do more than refer to these previous descriptions as being equally applicable to the species under consideration.

In this species of trypanosome also, as in *T. gambiense*, it is only a small percentage of the flies fed on an infected animal which become infected. In one series of *T. gambiense* this was 8 per cent.* In this species the experiments with laboratory-bred flies was 8.7 per cent., with wild flies 9 per cent. Just as in *T. gambiense*, the development takes place in the alimentary tract and salivary glands, and not in the proboscis.

The Trypanosomes found in the Salivary Glands.

In the trypanosome causing disease in man in Nyasaland, as in *T. gambiense*, the crux of the whole matter is the invasion of the salivary glands. After a certain number of days—in this species from 14 to 31—the trypanosomes reach the salivary glands and the fly becomes infective.

Plate 7, figs. 3-28, represent the various stages in the development of this trypanosome in the salivary glands. Figs. 1 and 2 are trypanosomes from the proventriculus; these represent the dominant intestinal type, from which the salivary-gland types arise. It is still a matter of speculation as to how they gain access to the glands, but, as described in a former paper,† there is no doubt they are often thrown forward into the proboscis during, or just in the act of feeding, and may, under these conditions, be drawn into the hypopharynx and so reach their destination. These proventricular forms, however, have never been actually seen by the Commission in the hypopharynx. Figs. 3-11 are forms found in the salivary glands. Many of these are crithidial in type and occur in numbers. Figs. 12-14 are what appear to be encysted forms. Figs. 16-21 are "blood forms" and occurred in large numbers in the same preparation as the crithidial type shown in figs. 3-8. Figs. 22-28 are "blood forms" which were thrown out on to a cover-glass by a living infective fly. The preparation was beautifully clear, each individual trypanosome standing out distinctly. Fig. 23 is from the same preparation and has the appearance of a small bunch or clump of "blood forms" in the act of breaking apart.

Conclusions.

1. The trypanosome causing Disease in Man in Nyasaland belongs to the same group as *T. gambiense*, the development taking place in the alimentary tract and salivary glands, not in the proboscis, of the fly.

2. The percentage of flies which become infected is the same as in *T. gambiense*, 8 per cent.

* "Proc." Roy. Soc., B, vol. 83 (1911), p. 514; also Reports of the Sleeping Sickness Commission, No. XI., p. 34.

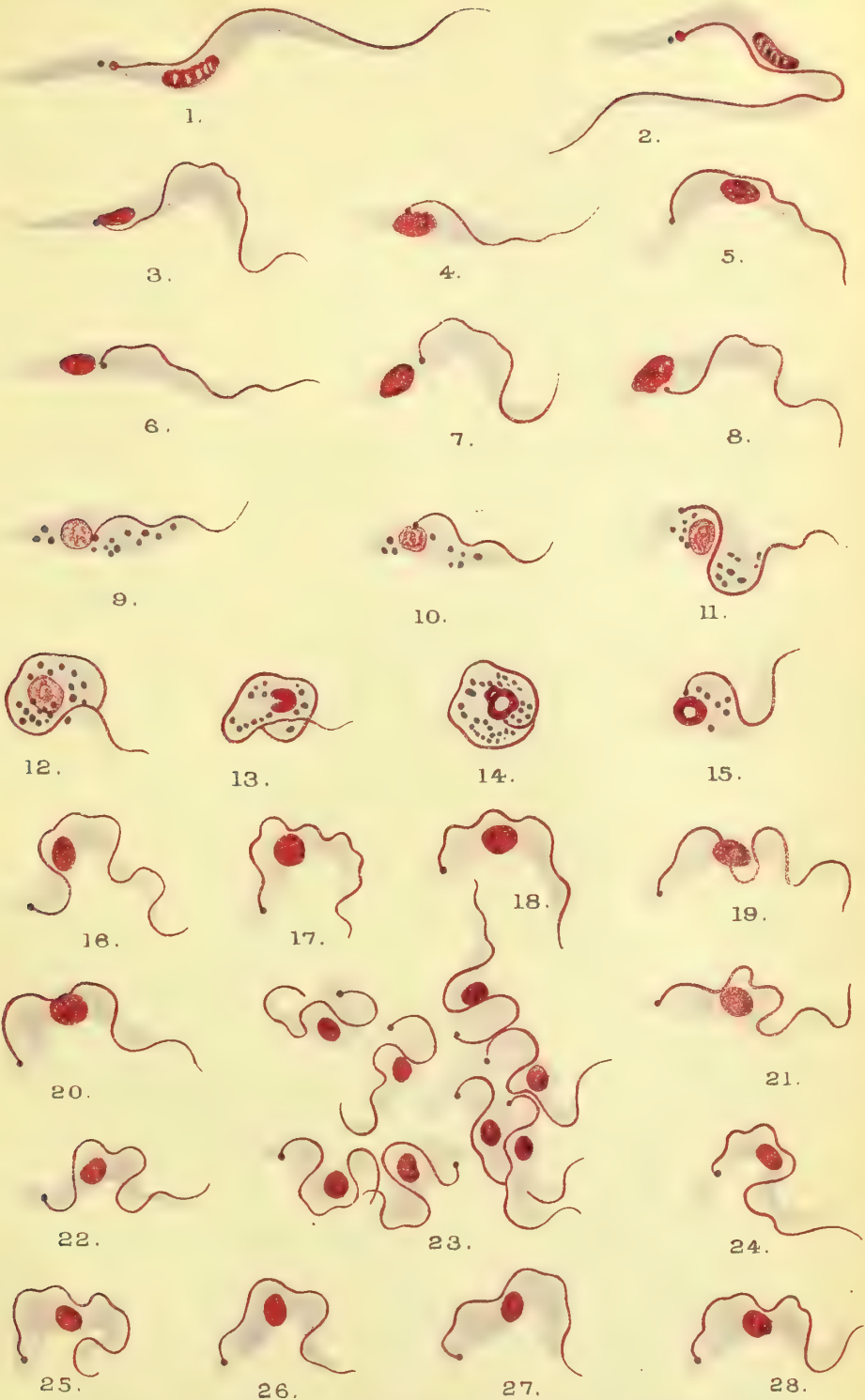
† "Proceedings" of the Royal Society, 87 (1913), p. 65; also Reports of the Sleeping Sickness Commission, No. XV., p. 34.

3. The percentage of flies which become *infective* is about 1 per cent.
4. The length of time which elapses before a fly becomes infective varies from 14 to 31 days, average 23 days.
5. The infective type of trypanosome in the salivary glands—corresponding to the final stage of the cycle of development—is similar to the short and stumpy form found in the blood of the vertebrate host.

DESCRIPTION OF PLATE 7.

- Figs. 1-2.—Trypanosomes from proventriculus. These represent the dominant intestinal type.
- Figs. 3-8.—Trypanosomes taken from a preparation of the salivary gland of an infective fly. Many of these are crithidial in type, *e.g.*, figs. 6, 7, and 8.
- Figs. 9-15.—Other forms seen in the salivary glands. Figs. 12-14 have the appearance of being encysted.
- Figs. 16-21.—The fully developed "blood forms." Without these the fly is non-infective. These were drawn from the same preparation as figs. 3-8.
- Figs. 22-28.—Trypanosomes ejected by a living infective *G. morsitans* on attempting to feed through a cover-glass. Fully developed "blood forms."

Stained Giemsa. × 2000.



Trypanosome Causing Disease in Man in Nyasaland.

Development in Glossina morsitans.

X 2000.

XXIX.—DEVELOPMENT IN *GLOSSINA MORSITANS* OF THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN. THE ZULULAND 1913 STRAIN.* STRAIN XXV.

In previous papers† the morphology of this trypanosome and its action on animals were described. The chief object of this paper is to compare the development of this trypanosome with that of the Trypanosome causing Disease in Man in Nyasaland—the *T. brucei* vel *rhodesiense*; the Human Strain. The development of the latter has already been described.‡ It will, therefore, only be necessary here to present the various data in the form of tables and figures, which can then be compared with similar tables and figures in the previous paper.

The Development of T. brucei, Zululand Strain XXV., 1913, in G. morsitans.

Seven experiments were made with laboratory-bred flies. Three of these were positive and four negative. Two experiments were also made with wild flies, both of which were positive.

Table CXIV.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1913						
Feb. 11	1857	58	—	1		
„ 17	1909	50	+	10	21	?
Mar. 11	1988	45	+	20	13	84°F. (29°C.)
„ 17	1996	55	—	4		
April 25	2111	50	—	3		
May 26	2188	30	—	1		
June 23	2188A	20	+	8	14	84°F. (29°C.)

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 526.

† "Proc. Roy. Soc.," B, vol. 87, 1914, pp. 493 and 511; also this Report, pp. 71 and 119.

‡ "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 516; also this Report, p. 135.

Three hundred and eight flies were used and 47 infected flies were found—15.3 per cent.

It is difficult or impossible to explain the difference in the ratio of infected flies. Experiment 1857 has only one infected fly in 58; Experiment 1988, 20 in 45. There is no record as to whether Cage 1857 was kept in the incubator or not, but it is to be presumed that it was, as was the habit at that date. From Table CXVI. it will be seen that the flies in Experiment 1988 were fed for eight days on an infected dog, monkey, and goat. It is possible that this had something to do with the high rate of infection, but it is impossible to say with certainty. The scarcity of laboratory-bred flies made it out of the question to pursue this line of inquiry. Experiment 2188 has also only one infected fly in 30, but this is capable of explanation. Experiments 2188 and 2188A were carried out for the sake of economy with the same cage of flies. It having become evident (see Table CXVII.) that the flies, after their first feeding on an infected rat, had failed to infect Monkey 2203, the 20 remaining flies were again fed on an infected guinea-pig, with the result that eight of them became infected.

Table CXV.—Wild Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1913.						
July 22	2309	50	+	0	34	84°F. (29°C.)
„ 26	2313	50	+	5	24	84°F. (29°C.)

In Experiment 2309 none of the flies were dissected, hence no infected flies were found. In Experiment 2313, only 21 flies out of 50 were dissected. These experiments are tabulated here as they give the number of days before the flies became infective, and thus afford data as to the length of time the cycle of development runs in the fly.

Details of the Five Positive Experiments.

The following table gives the principal details in the carrying out of the five positive experiments. The first three were carried out with laboratory-bred flies, the last two with wild flies.

It would appear from these five experiments that an average period of 21 days elapses before the cycle of development of *T. brucei*, Zululand, 1913, is complete in *G. morsitans* and the fly becomes infective.

Table CXVI.

Expt.	Day of expt.	Procedure.	Remarks.
1909	1—9 10 11—29	Flies fed on infected monkey Starved Fed on clean monkey 1970	Trypanosomes appeared in blood of monkey 1970 on the 28th day
1988	1—8 9 10—22 23 24—29	Flies fed on infected dog, monkey, and goat Starved Fed on clean dog 2007 Starved Fed on clean monkey 2058	Trypanosomes appeared in blood of dog 2007 on the 20th day Monkey 2058 showed trypanosomes on the 30th day
2188A	1—9 10 11—22 23 24—27	Flies fed on infected guinea-pig Starved Fed on clean dog 2254 Starved Fed on clean monkey 2298	Trypanosomes appeared in blood of dog 2254 on the 21st day Monkey 2298 showed trypanosomes on the 32nd day
2309	1—7 8 9—42	Flies fed on infected dog Starved Fed on clean monkey 2316	Trypanosomes appeared in blood of monkey 2316 on the 41st day
2313	1—4 5 6—15 16 17—31	Flies fed on infected monkey Starved Fed on clean monkey 2318 Starved Fed on clean dog 2361	Trypanosomes appeared in blood of dog 2361 on the 31st day Monkey 2318 never showed trypanosomes

Details of the Four Negative Experiments.

The following table shows the method of procedure in carrying out the four negative experiments. In each of them laboratory-bred flies were used:—

Table CXVII.

Expt.	Day of expt.	Procedure.	Remarks.
1857	1—8 9 10—60	Flies fed on infected monkey Starved Fed on clean monkey 1941	One infected fly found on the 62nd day
1996	1—9 10 11—55	Flies fed on infected monkey Starved Fed on clean monkey 2031	Four infected flies found
2111	1—8 9 10—14 15 16—30 31 32—43	Flies fed on infected dog Starved Fed on clean dog 2100 Starved Fed on clean monkey 2125 Starved Fed on clean dog 2189	Three infected flies found
2188	1—8 9 10—27	Flies fed on infected rat Starved Fed on clean monkey 2203	One infected fly found on the 21st day

Result of the Dissection of the Infected Flies.

Table CXVIII.—Laboratory-bred Flies. Positive Experiments.

Expt.	Time, days.	Proboscis.		Proventri- culus.	Crop.	Fore- gut.	Mid- gut.	Hind- gut.	Salivary glands.
		Labial cavity.	Hypo- pharynx.						
1909	33	—				+			—
1909	34	—		+		++	++	++	—
1909	35	—		—		++	++	++	++
1909	36	—		++		++	++	++	++
1909	37	—				+	+		—
1909	43	—				++	++		—
1909	47	—		+			+		—
1909	50	—					+		—
1909	51	—	—			++	++		++
1909	57	—		—			+		—
1988	22	—	—		++	++	++	++	—
1988	23	—	—	—	—	—	+		—
1988	30	—	—	+		+			—
1988	30	—	—	—			+		—
1988	31	—	—	—			+		—
1988	31	—	+	+	+	+		+	++
1988	31	+	—	+		+		+	—
1988	33	—	—				+		—
1988	34	—	—				+		—
1988	34	—		+		+		+	+
1988	34	—	—				+		—
1988	34	—	—				+		—
1988	34	—	—				+		—
1988	34	—	—				+		—
1988	34	—	—	+			+		—
1988	35	—	—	—			+		—
1988	36	—	—	+		+		+	—
1988	37	—	—			+		+	—
1988	37	—	—	+		+		+	—
2188A	24	—	—				+		—
2188A	24	—	—				+		—
2188A	29						+		—
2188A	32						+		—
2188A	32						+		—
2188A	32						+		—
2188A	32					+		+	++
2188A	32						+		—

In Experiment 1909, 10 infected flies were found. Three of these had the salivary glands swarming with trypanosomes; in none was the labial cavity or hypopharynx found to contain flagellates.

In Experiment 1988, 20 infected flies were found. Two had an invasion of the salivary glands. In one it is noted that a few active trypanosomes were seen in the labial cavity, and in one that a few "blood forms" were seen in the hypopharynx.

In Experiment 2188A, eight infected flies were found, one of which had the salivary glands swarming with trypanosomes. Parts of these glands injected into Rats 2311 and 2312 gave, in both cases, a positive result.

Table CXIX.—Laboratory-bred Flies. Negative Experiments.

Expt.	Time, days.	Proboscis.		Proventri- culus.	Crop.	Fore- gut.	Mid- gut.	Hind- gut.	Salivary glands.
		Labial cavity.	Hypo- pharynx.						
1857	62	—	—	—			+		—
1996	23					++	++	++	—
1926	43			—	—	+	+	+	—
1906	57	—	—	—		++	++	++	—
1996	57						++		—
2111	12	—	—	—	—	—	+	+	—
2111	29	—	—	+	+	++	++	++	—
2111	40	—	—				+		—
2188	21					—	+	+	—

In none of the negative experiments was an infection of the salivary glands found. Nine infected flies were dissected, but not one of these had passed into the infective stage. No parasites were found in the proboscis at any time.

From a consideration of these tables it will be seen that this strain of *T. Brucei*, Zululand, 1913, belongs to the same group as *T. gambiense* and the Trypanosome causing Disease in Man in Nyasaland as far as their development in *G. palpalis* and *G. morsitans* is concerned. This development takes place in the intestine of the fly, and, after a varying number of days, passes forward into the salivary glands, where the final stage in the cycle is reached—the infective or “blood forms.” In this group the parasites are never found fixed in the labial cavity as in the *pecorum* and *vivax* groups.

The Type of Trypanosome found in the Infected Flies.

Plate 8 represents the developmental forms of *T. brucei*, Zululand, 1913, found in the proventriculus and salivary glands of *G. morsitans*. A description of the various types found in the different parts of the alimentary tract is not considered necessary, as they are identical with those found in the development of *T. gambiense* in *G. palpalis*, which have already been fully described.*

* “Proc. Roy. Soc.,” B, vol. 83 (1911), p. 513, and Reports of the Sleeping Sickness Commission, No. XI., p. 34.

Figs. 1 and 2 are two long trypanosomes from the proventriculus. Figs. 3-6 are the same, but they were extruded on to a cover-glass by a living non-infective fly, and probably came through the labial cavity from the proventriculus. Some of them may possibly have come from the hypopharynx, and, in that case, they may have been proventricular forms on their way to infect the salivary glands. Figs. 7-20 are various types found in the salivary glands—crithidial, encysted, and immature “blood forms.” Figs. 21-28 represent the finished product—the infective or “blood forms.”

If this plate is compared with that representing the developmental forms of the Trypanosome causing Disease in Man in Nyasaland* in the proventriculus and salivary glands of *G. morsitans*, the extraordinary likeness between the two is at once evident, and is another argument in favour of these two strains being identical.

Conclusions.

1. *T. brucei*, Zululand, 1913, belongs to the same group as *T. gambiense* as regards its cycle of development in the tsetse fly.

2. It has been shown that the Trypanosome causing Disease in Man in Nyasaland also belongs to the same group.

3. The cycle of development of the Nyasaland and Zululand trypanosomes in *G. morsitans* is so marvellously alike that it affords another reason for believing in the identity of these two trypanosomes.

DESCRIPTION OF PLATE 8.

Figs. 1 and 2.—Trypanosomes from proventriculus.

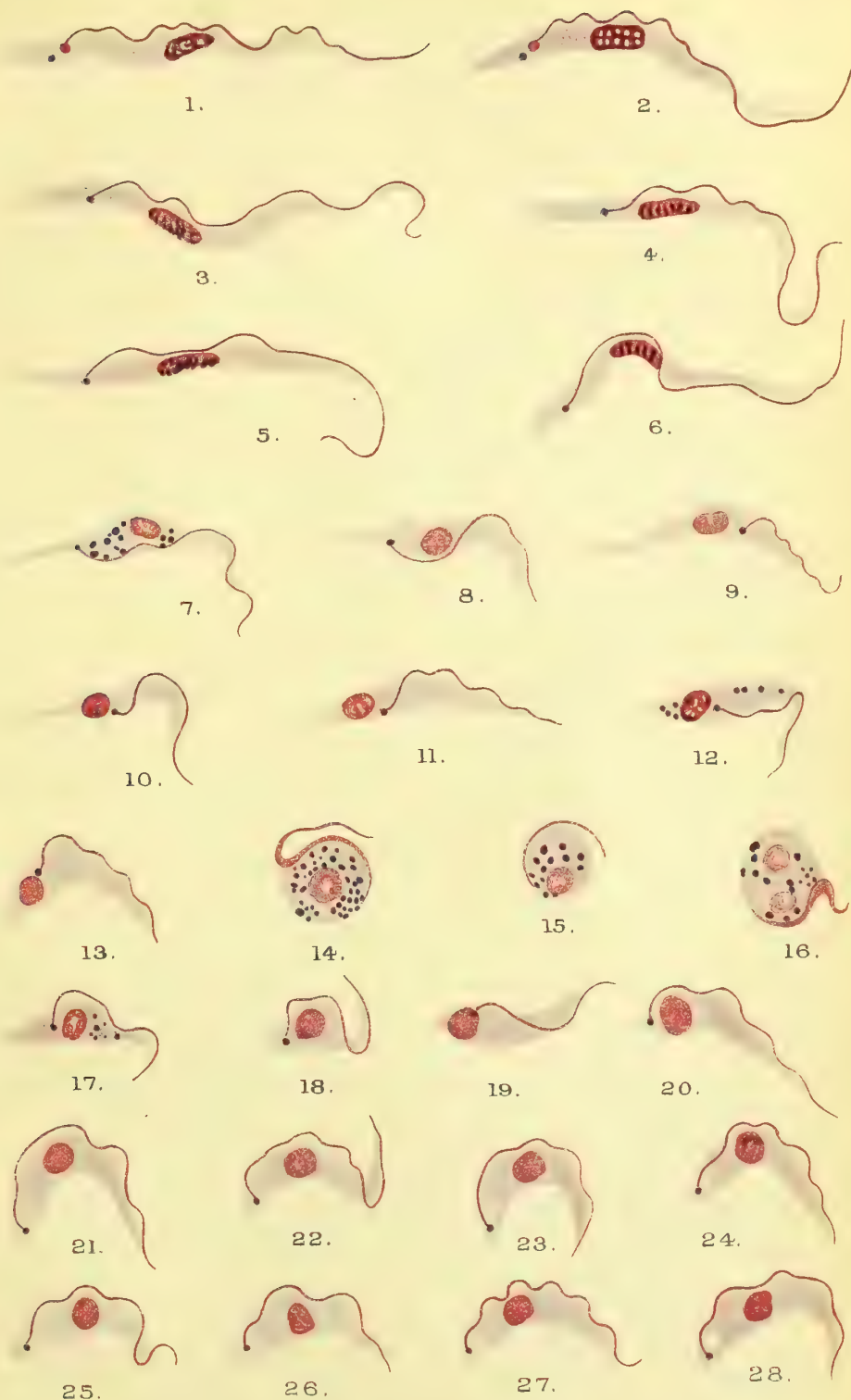
Figs. 3-6.—Proventricular types which were extruded on to a cover-glass by a living non-infective fly.

Figs. 7-20.—Crithidial, apparently encysted, and immature “blood forms” found in the salivary glands.

Figs. 21-28.—The final stage in the salivary glands—the infective or “blood forms.”

Stained Giemsa. × 2000.

* “Proc. Roy. Soc.,” B, vol. 87, 1914, Plate 24; also this Report, Plate 7



Trypanosoma brucei. Zulularud 1913.

Development in Glossina morsitans.

X 2000.

XXX.—DEVELOPMENT IN *GLOSSINA MORSITANS* OF THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHO-DESIENSE*—CAUSING DISEASE IN MAN IN NYASALAND. THE NATURALLY-INFECTED DOG STRAIN.*

In previous papers† the morphology and action on animals of this strain of trypanosome were described. In this a short account of its development in *Glossina morsitans* is given, in order to compare it with the development of the Human Strain of the Trypanosome causing Disease in Man in Nyasaland.‡

It is to be regretted that more material is not available, but, scanty as it is, there is enough to show that this strain develops in the alimentary tract and salivary glands of *Glossina morsitans* in the same way as the *Trypanosoma brucei* and *gambiense* group. The Commission aimed at having five positive experiments in every series of transmission experiments, but in this case failed. The failure was principally due to the difficulty of procuring laboratory-bred flies, and also to the fact that this strain of trypanosome does not readily develop in *Glossina morsitans*.

The Development of the Naturally-Infected Dog Strain in Glossina Morsitans.

Eleven experiments were made with laboratory-bred flies. Two were positive and nine negative.

Table CXX.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Expt. positive or negative.	No. of flies dissected.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1912.							
Sept. 7	1257	25	—	25	0		84°F. (29°C.)
Oct. 23	1499	26	—	26	1		"
Dec. 3	1668	10	—	10	0		"
1913.							
Jan. 13	1753	50	—	18	0		84°F. (29°C.)
Mar. 24	2018	55	—	18	2		"
April 7	2067	30	+	30	3	24	"
May 9	2018A	40	—	36	7		"
June 13	2226	40	—	8	1		"
July 21	2303	40	—	29	0		"
Aug. 30	2394	40	+	0	0	58	"
Nov. 12	2433	20	—	15	0		"

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 213.

† "Proceedings" of the Royal Society, B, vol. 88, pp. 111 and 130; also this Report, pp. 88 and 125.

‡ "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 516; also this Report, p. 135.

Three hundred and seventy-six flies were used and 14 were found infected—3·7 per cent. This small percentage is partly due to the fact that in some of the experiments few or none of the flies were dissected. There is the same discrepancy to be noted here as in *Trypanosoma brucei*, Zululand Strain XXV., 1913. In some of the experiments, not a single infected fly was found, whereas in Experiment 2018A there were seven in a cageful of 36.

Details of the Two Positive Experiments.

The following table gives the details in the carrying out of the two positive experiments. They were both carried out with laboratory-bred flies.

Table CXXI.

Date.	Day of expt.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1913.		Experiment	2067.		
April 7					
" 8	1	30 flies fed on infected rat 2024			
" 9	2				
" 10	3				
" 11	4				
" 12	5				
" 13	6	Starved			
" 14	7				
" 15	8				
" 16	9				
" 17	10				
" 18	11				
" 19	12				
" 20	13				
" 21	14				
" 22	15				
" 23	16				
" 24	17				
" 25	18				
" 26	19	Fed on clean dog 2054	+		Trypanosomes appeared in blood of dog 2054 on the 31st day
" 27	20				
" 28	21				
" 29	22				
" 30	23				
May 1	24				
" 2	25				
" 3	26				
" 4	27				
" 5	28				
" 6	29				
" 7	30				
" 8	31				
" 9	32	Starved			
" 10	33	Fed on clean monkey 2131	+		Trypanosomes appeared in blood of monkey 2131 on the 42nd day
" 11	34				
" 12	35				
" 13	36	Starved			

Table CXXI. (continued).

Date.	Day of expt.	Procedure.	Result.		Remarks.
			Posi- tive.	Nega- tive.	
1913.		Experiment	2067—	<i>continued.</i>	
May 14	37	Fed on clean guinea- pig 2145		—	
" 15	38				
" 16	39				
" 17	40				
" 18	41				
" 19	42	Starved			
" 20	43				
" 21	44				
" 22	45	Fed on clean duiker 2059	+		Trypanosomes appear- ed in blood of duiker 2059 on the 60th day
" 23	46				
" 24	47				
" 25	48	Starved			
" 26	49				
" 27	50				
" 28	51	Fed on clean monkey 2184	+		Trypanosomes appear- ed in blood of monkey 2184 on the 63rd day
" 29	52				
" 30	53				
" 31	54				
All flies dissected and three found infected					
Experiment 2394.					
1913.					
Aug. 20	1—6	40 flies fed on infected	+		Trypanosomes appear- ed in blood of dog 2404 on the 65th day. No flies dissected
to 26		rat 2389			
Aug. 21	7	Starved			
" 22	8—65	Fed on clean dog 2404			
to					
Oct. 24					

Experiment 2067 was successful, as all the animals the flies fed on became infected, with the exception of the guinea-pig, and it will be remembered that the guinea-pig was found to be refractory to this strain.

Experiment 2394 also infected a dog, but as none of the flies were dissected none were found infected.

From these two experiments it would appear that a period of from 24 to 58 days may elapse before the cycle of development of the Naturally-Infected Dog Strain is complete in *Glossina morsitans* and the fly becomes infective.

Details of the Nine Negative Experiments.

The following table shows the method of procedure in carrying out the nine negative experiments. In each of them laboratory-bred flies were used.

Table CXXII.

Expt.	Day of expt.	Procedure.	Remarks.
1257	1—5 6 7—44	25 flies fed on infected dog 690 Starved Fed on clean dog 1313	All flies dissected; all negative
1499	1—3 4 5—48	26 flies fed on infected rat 1218 Starved Fed on clean dog 1500	All flies dissected; one found infected
1668	1—3 4—5 6—23	10 flies fed on infected monkey 1630 Starved Fed on clean monkey 1670	All flies dissected; all negative
1753	1—3 4 5—40	50 flies fed on infected monkey 1534 Starved Fed on clean monkey 1778	18 flies dissected; all negative
2018	1—8 9 10—32 31 32—45	55 flies fed on infected rats 1985 and 2023 Starved Fed on clean monkey 2056 Starved Fed on clean dog 2112	18 flies dissected; two infected
2018a	1—8 9 10—35	40 flies fed on infected dog 2054 Starved Fed on clean dog 2172	36 flies dissected; seven found infected
2226	1—6 7 8—37	40 flies fed on infected rat 2214 Starved Fed on clean dog 2233	8 flies dissected; one infected
2303	1—2 3—13 14 15—44	40 flies fed on infected duiker 2059 Fed on infected rat 2280 Starved Fed on clean dog 2319	29 flies dissected; all negative
2433	1—3 4 5—38	20 flies fed on infected rat 2425 Starved Fed on clean dog 2435	20 flies dissected; all negative

Result of the Dissection of the Infected Flies.

Table CXXIII.—Laboratory-bred Flies. Positive Experiments.
Time, in Days, means the Number of Days which elapsed between the First Infective Feed and the Death and Dissection of the Fly.

Expt.	Time, days.	Proboscis.	Proventriculus.	Crop.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
2067	42	—			+	+	+	—
2067	50	—	++		+	+	—	++
2067	51				+	+		—

In Experiment 2067, 30 flies were used; all were dissected and three found infected—10 per cent. This experiment was carefully carried out from beginning to end, and yet only one fly was found with invasion of the salivary glands. This one fly seems to have done all the mischief, infecting one after the other a dog, monkey, antelope, and, finally, another monkey. There is some doubt about this last monkey, as the fly died 50 days after its first infective feed, and the monkey had only come into use the day before. This particular fly, however, is reported not to have fed on that day, no fresh blood having been found in its intestine. It may be that it attempted to feed and so infected the monkey, but was unable to draw blood. If this fly did not infect the last monkey, it is difficult to explain its infection, as all the remaining flies were dissected on the 54th day and all found to be negative.

In Experiment 2394, none of the flies were dissected.

Table CXXIV.—Laboratory-bred Flies. Negative Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Crop.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
1499	11	—	++	—	++	+		—
2018	24	—	++		++	++	++	—
2018	30	—			+	+		—
2018A	13	—			++	++	+	—
2018A	14	—			++	++	+	—
2018A	15	—	—		+	+		—
2018A	17	—			++	++	+	—
2018A	18	—			++	++	++	—
2018A	35	—			+	+		—
2018A	35	—			+	+		—
2226	32				++	++	+	—

One hundred and eighty-five flies were dissected and 11 found infected—5.9 per cent. In none was there found any development in the proboscis, nor invasion of the salivary glands.

From an examination of these tables it will be admitted, in spite of the paucity of the material, that the Naturally-Infected Dog Strain belongs to the same group as *Trypanosoma gambiense* and *Trypanosoma brucei* vel *rhodesiense* in regard to its mode of development in *Glossina morsitans*.

The Type of Trypanosome found in the Infected Flies.

A number of drawings of the developmental forms of the Naturally-Infected Dog Strain of trypanosome was made from the alimentary tract and salivary glands of the infected tsetse flies. In the intestine the same type of trypanosome was found which has already been described and figured in previous papers.* In the only infected fly which showed a development in the salivary glands, the trypanosomes were described in the living unstained preparations as being exceedingly numerous, small, and active. In the stained preparations the trypanosomes were seen to be typical "blood forms" and absolutely identical to those figured in the development of the Trypanosome causing Disease in Man in Nyasaland,† and also, in that of *Trypanosome brucei*, Zululand, 1913.‡ It is therefore unnecessary to figure them again.

Conclusion.

The trypanosome of the Naturally-Infected Dog Strain belongs to the same group as *Trypanosoma gambiense* and *Trypanosoma brucei* vel *rhodesiense*, the Trypanosome causing Disease in Man in Nyasaland, and is probably merely a weak strain of the latter species.

* "Proceedings" of the Royal Society, B, vol. 83, 1911, p. 513, and Reports of the Sleeping Sickness Commission, No. XI., p. 34.

† "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 516; also this Report, p. 144.

‡ "Proceedings" of the Royal Society, B, vol. 87, 1914, p. 526; also this Report, p. 150.

XXXI.—DEVELOPMENT IN *GLOSSINA BREVIPALPIS* OF
THE TRYPANOSOME—*T. BRUCEI* VEL *RHODESIENSE*
—CAUSING DISEASE IN MAN IN NYASALAND.*

Transmission Experiments.

Several experiments were carried out with *Glossina brevipalpis*, to ascertain if this species of tsetse fly can act as a carrier of the various pathogenic trypanosomes found in Nyasaland.

These experiments were made, not with laboratory-bred but with wild flies, and this, of course, takes away much of their value. It was found impossible, on account of the distance from the Lake-shore and the scarcity of flies, to attempt the breeding of *Glossina brevipalpis*, in order to obtain laboratory-bred flies.

The species or strains of trypanosomes experimented with were:

(1) *Trypanosoma brucei* vel *rhodesiense*, the Trypanosome causing Disease in Man in Nyasaland; (2) *Trypanosoma brucei*, Zululand, 1913; (3) *Trypanosoma pecorum*; and (4) *Trypanosoma capræ*.

1. *The Development of Trypanosoma brucei vel rhodesiense in Glossina brevipalpis.*

Feeding wild Glossina brevipalpis First on Animals Infected with the Trypanosome causing Disease in Man in Nyasaland and then on Healthy Animals, to discover if this Species of Trypanosome passes through a Cycle of Development in this Species of Tsetse Fly.

Table CXXV.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of flies dissected.	No. of infected flies found.	No. of days before flies became infective.
1913.						
Mar. 6	1986	2	—	1	0	—
" 26	2062	10	—	6	0	—
June 2	2201	70	—	8	2	—
" 9	2213	60	—	19	2	—
" 21	2232	50	—	18	4	—
July 23	2310	40	—	1	0	—

Two hundred and thirty-two wild *Glossina brevipalpis* were used in six experiments, but in no case with a positive result. Only 53 flies were dissected and eight infected flies found. If all had been dissected probably about 30 to 40 infected flies would have been found.

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 24.

Details of the Six Negative Experiments.

Table CXXVI.

Expt.	Day of expt.	Procedure.	Remarks.
1986	1	Flies fed on infected guinea-pig 1658	Only two flies used; one escaped and the other died on the 15th day of the experiment; on dissection it was found negative
	2-4	Starved	
	5-15	Fed on clean guinea-pig 1907	
2062	1-4	Flies fed on infected monkey 970	Ten flies used. Six were dissected; all negative
	5	Starved	
	6-28	Fed on clean dog 2041	
2201	1-6	Flies fed on infected monkey 2156	Seventy flies used. Only eight were dissected; two found infected, one with <i>T. grayi</i> , the other with a pathogenic type of trypanosome
	7	Starved	
	8-33	Fed on clean dog 2211	
2213	1-12	Flies fed on infected monkey 2151	Sixty flies used. Two found infected; only 19 dissected
	13-14	Starved	
	15-44	Fed on clean dog 2237	
2232	1-6	Flies fed on infected monkey 2152	Fifty flies used. Four found infected; 18 flies dissected. The four infected flies showed infection of the gut alone
	7-9	Starved	
	10-42	Fed on clean dog 2244	
2310	1-3	Flies fed on infected monkey 1792	Forty flies used. Only one fly dissected; negative
	4-5	Starved	
	6-39	Fed on clean dog 2315	

Result of the Dissection of the Infected Flies.

As will be seen from Table CXXV., eight infected flies were found among the *Glossina brevipalpis* which had fed on animals infected with the Trypanosome causing Disease in Man in Nyasaland. The following table gives the result of the dissection of these eight flies:—

Table CXXVII.

Expt.	Time, days.	Proboscis.	Alimentary tract.	Salivary glands.
2201	30	—	+	—
"	37	—	+	—
2213	33	—	+	+
"	46	+	++	+
2232	37	—	+	—
"	37	—	+	—
"	44	—	+	—
"	44	—	+	—

In Experiment 2201 there were two infected flies found. One of these appeared to be infected with *Trypanosoma grayi*, the other with a trypanosome of a pathogenic type.

In Experiment 2213 there were also two infected flies. In one the development was restricted to the proboscis and the alimentary tract; in the other the salivary glands as well as the intestine were found to be swarming with trypanosomes. This fly, which was dissected 33 days after it had fed on an infected monkey, had the whole lumen of the glands filled with active motile trypanosomes, which came pouring out of the broken end of the glands. It was thought at the time that these must be infective forms of the Trypanosome causing Disease in Man in Nyasaland, but a part of the salivary glands and contents of gut injected into a white rat failed to infect it. In spite of this negative experiment, however, it is probable that this represents a true development of the Nyasaland trypanosome in *Glossina brevipalpis*, the development not having reached the infective stage. A fuller description of the morphology of these salivary forms will be given under the next heading. It may be noted here that salivary glands infected by *Trypanosoma brucei* vel *rhodesiense*, or by *Trypanosoma brucei*, Zululand, 1913, seem to be more crowded with trypanosomes than in the corresponding infection of *Glossina palpalis* by *Trypanosoma gambiense*. The salivary glands in the former case appear to be swollen and bursting with the flagellates.

In Experiment 2232 there were four infected flies found, but in none was there any invasion of the salivary glands.

Morphology of the Trypanosomes found in the Salivary Glands of a Wild Glossina brevipalpis which had fed on a Monkey Infected with the Trypanosome causing Disease in Man in Nyasaland.

This fly, as described above, was found in Experiment 2213, and failed to infect the clean Dog 2237, which it had been fed upon. Part of the salivary glands and contents of the intestine also failed to infect Rat 2234. It would appear, then, that this fly had not yet reached the infective stage.

It is now proposed to describe the different forms of the trypanosomes found in the salivary glands of this fly somewhat in detail, and to bring forward a theory in regard to a stage which seems to occur in the final development of this trypanosome in the salivary glands. The different forms and apparent stages in their development could be more easily made out in *Glossina brevipalpis* than in *Glossina morsitans*, on account of its greater size. On Plate 9 this evolution of the Trypanosome causing Disease in Man in Nyasaland in the salivary glands is represented.

Figs. 1 and 2 represent the long, slender developmental forms of trypanosomes found in the intestine of the fly, from the mid-gut to the proventriculus. It is this type of trypanosome which invades the salivary glands.

Fig. 3 shows the change in shape which the intestinal forms undergo on entering the salivary glands. The posterior extremity

lengthens somewhat and the micronucleus and flagellum pass forward. This appears to be the commencement of the change to the crithidial type.

Fig. 4 represents the fully developed crithidial form of the parasite. The micronucleus and flagellum have passed further forward until they lie anterior to the nucleus. The anterior portion of the parasite has broadened out; the posterior has become attenuated.

Fig. 5, the parasite is still crithidial in form. The anterior half has become still broader, the posterior half elongated and further attenuated.

Fig. 6, the attenuated posterior portion has shortened.

Figs. 7, 8, and 9, represent further stages in the evolution. The long attenuated posterior extremity has disappeared and the parasite has become contracted and thickened.

Fig. 10 shows the last phase of the crithidial stage. Here the parasite has assumed a rounded form and the flagellum is folding on itself.

Fig. 11 represents a group of parasites in the encysted stage. In this form they are found massed together in the lumen of the salivary glands.

Fig. 12, in this group the encysted forms are just unfolding.

Fig. 13 shows a single encysted form breaking open. The micronucleus is now posterior to the nucleus; the crithidial has become the trypanosomal.

Figs. 14, 15, 16, and 17, demonstrate further stages in the unfolding of the encysted form. The parasite is now assuming a trypanosome shape.

Figs. 18 and 19 show the fully developed salivary-gland form of the trypanosome. This constitutes a reversion to the "blood form" from which the cycle of development began and is the only infective form.

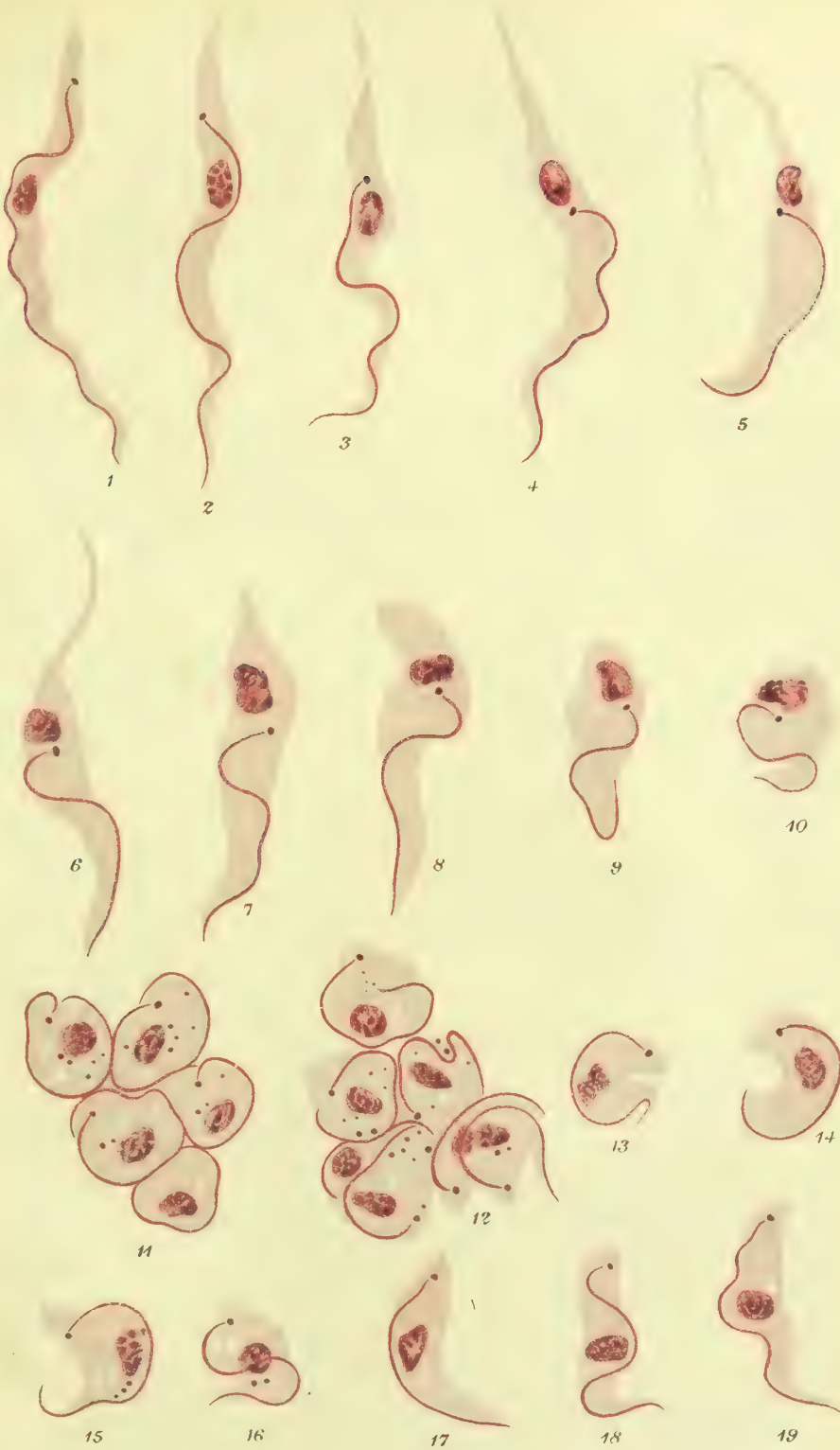
On comparing these figures with the developmental forms of the Trypanosome causing Disease in Man in Nyasaland in *Glossina morsitans*,* or with the same forms of *Trypanosoma brucei*, Zululand, 1913,† it will be apparent that in all probability this is a true development of this trypanosome in *Glossina brevipalpis*. It is true wild flies are being dealt with, but in this district it is only trypanosomes of this type which invade the salivary glands, so that *Trypanosoma pecorum*, *Trypanosoma simæ*, and *Trypanosoma capræ* are excluded.

Conclusion.

Glossina brevipalpis is capable of acting as a carrier of *Trypanosoma brucei*, the Trypanosome causing Disease in Man in Nyasaland.

* "Proceedings" of the Royal Society, B, vol. 87, 1914, Plate 24; and this Report, Plate 7.

† "Proceedings" of the Royal Society, B, vol. 87, 1914, Plate 25; and this Report, Plate 8.



*Trypanosome causing Disease in Man in Nyasaland
Development in Glossina brevipalpis.*

XXXII.—DEVELOPMENT IN *GLOSSINA BREVIPALPIS* OF THE TRYPANOSOME—*TRYPANOSOMA BRUCEI* VEL *RHODESIENSE*—CAUSING DISEASE IN MAN. THE ZULULAND 1913 STRAIN. STRAIN XXV.*

Feeding Wild Glossina brevipalpis First on Animals Infected with Trypanosoma brucei, Zululand Strain XXV., 1913, and then on Healthy Animals, to ascertain if this Species of Trypanosome passes through a Cycle of Development in this Species of Tsetse Fly.

Table CXXVIII.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of flies dissected.	No. of infected flies found.	No. of days before flies became infective.
1912.						
May 12	2130	80	—	10	1	—
July 1	2250	60	+	2	0	51?
„ 17	2299	60	—	0	0	—

Details of the Three Experiments.

Table CXXIX.

Expt.	Day of expt.	Procedure.	Remarks.
2130	1—2	Flies fed on infected monkey	Eighty flies used. One found infected. Only 10 dissected
	3	Starved	
	4—35	Fed on clean dog 2142	
2250	1—4	Flies fed on infected dog	Sixty flies used. Only two dissected; both negative Dog 2276 showed trypanosomes on the 58th day
	5—6	Starved	
	7—60	Fed on clean dog 2276	
2299	1—10	Flies fed on infected dog	Sixty flies used. None dissected
	11	Starved	
	12—61	Fed on clean dog 2314	

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 23

Two hundred wild *Glossina brevipalpis* were used in three experiments—one positive, two negative. Only 12 flies were dissected, one of which was found to contain in the intestine, long, ribbon-like trypanosomes of apparently a pathogenic type, but it was not possible to place it. It is to be regretted that all the flies had not been dissected as was the rule in these transmission experiments.

Dog 2276 became infected 58 days after the flies had fed on the infected animal. This would point to the development of *Trypanosoma brucei*, Zululand, in the flies. If there had been a fly in the cage naturally-infected with the Nyasaland strain, then the animal fed upon ought to have shown trypanosomes in its blood earlier than 58 days. It may, therefore, be held as highly probable that Dog 2276 was infected by a *Glossina brevipalpis* in which a development of *Trypanosoma brucei*, Zululand Strain XXV., 1913, had taken place.

Conclusion.

Glossina brevipalpis is capable of acting as a carrier of *Trypanosoma brucei*, Zululand Strain XXV., 1913.

XXXIII.—SERUM EXPERIMENTS.

The action of Human Serum on Trypanosoma brucei, Zululand Strain, 1913.

Experiment 2025.

The action of human serum on *Trypanosoma brucei*, Zululand Strain, was tested in Experiment 2025, in which 0.5 c.c. of human serum was allowed to act upon 10 c.cm. of blood containing that trypanosome, for five minutes at room temperature, the mixture then being injected into a rat intraperitoneally.

Trypanosomes appeared in this rat's blood on the 11th day, whereas the control rat showed trypanosomes on the 3rd day, showing that human serum has an inhibitory action on *Trypanosoma brucei*, Zululand Strain.

The action of Human Serum on Trypanosoma brucei, Zululand Strain, 1913, compared with its action on Trypanosoma brucei vel rhodesiense, Human Strain, Nyasaland.

Experiment 2096.

In this experiment, the action of human serum on *T. brucei*, Zululand Strain, and on *T. brucei vel rhodesiense*, Human Strain, Nyasaland, was contrasted.

The procedure was the same as in the preceding experiment. Ten c.cm. of blood containing *T. brucei*, Zululand Strain, were employed, and three lots of *T. brucei vel rhodesiense*, Human Strain, Nyasaland-infected blood, one of 10 c.cm. and two of 20 c.cm.

In each case *T. brucei* vel *rhodesiense*, Human Strain, Nyasaland, appeared in the blood on the fifth day, while *T. brucei*, Zululand Strain, had not appeared at the end of a month, when examinations were discontinued. In this case the serum appears to have rendered the latter trypanosome innocuous and to have had little or no action on the former.

This experiment may, of course, be used as an argument against the identity of *T. brucei*, Zululand Strain, and *T. brucei* vel *rhodesiense*, Nyasaland. But if the two are the same, it seems natural to expect that the strain which was taken from human blood would show more resistance to human serum than the other taken from wild game.

The action of Various Animal Sera on Trypanosoma brucei, Zululand Strain, 1913, and Trypanosoma brucei vel rhodesiense, Human Strain, Nyasaland.

Experiment 2460.

In Experiment 2460 the action of various sera on *T. brucei*, Zululand Strain, and *T. brucei* vel *rhodesiense*, Human Strain, Nyasaland, was compared.

The sera employed were those of the ox, guinea-pig, antelope, and man, a control being carried out with normal saline. The procedure was as in the two previous experiments, 10 c.cm. of blood being employed.

Trypanosomes appeared on the third day in the blood of the control rats, and also of the antelope-rat and the ox-rat for both strains; on the fourth day, in both guinea-pig-rats; and *T. brucei* vel *rhodesiense*, Human Strain, Nyasaland, appeared in the human-serum-rat on the fifth day. *T. brucei* Zululand, did not appear in the human-rat up to the 31st day, when examination was discontinued. This would show that guinea-pig's serum has a slight inhibitory action on both strains of trypanosomes, and human serum a slight inhibitory action on *T. brucei* vel *rhodesiense*, Human Strain, Nyasaland, and a much greater (or total) action on *T. brucei*, Zululand Strain.

This, like the last experiment, could be used as an argument to show that these two strains are distinct species, but the argument used above is also applicable here.

The action of Baboon's Serum on T. brucei vel rhodesiense (Human, Naturally-Infected Dog, and the Wild G. morsitans Strains).

Experiment 1747.

In Experiment 1747, 0.5 to 1 c.c. of baboon's serum was injected, subcutaneously, into a guinea-pig heavily infected with the Human Strain, and into two rats heavily infected with the Naturally-Infected Dog Strain and the Wild *G. morsitans* Strain.

In each case the trypanosomes completely disappeared from the blood in from two to four days, but then reappeared, and the disease pursued its course. Thus, baboon's serum appears to have a marked and equal antagonistic effect on these three strains. The baboon is naturally refractory to all three strains.

Human serum and baboon serum might, therefore, be distinguished from one another by their action on animals infected with the Human Strain. The former would have little or no effect, the latter a marked effect.

The action of Human Serum on T. brucei vel rhodesiense (Naturally-Infected Dog Strain and the Wild G. morsitans Strain).

Experiment 1776.

In this experiment similar subcutaneous injections of human serum (1 c.c.) were made into rats heavily infected with the Naturally-Infected Dog Strain and the Wild-Fly Strain.

The trypanosomes in the blood were much reduced in number, but less markedly than by baboon's serum. The action of human serum on the two trypanosomes would appear to be about the same.

This experiment may also be used as an argument that the Wild *G. morsitans* Strain belongs to another species than that found in man—the Human Strain. In other words, that the wild fly are infected with *T. brucei*, the natives with *T. rhodesiense*.

The Trypanolytic action of Immune Serum on Trypanosoma brucei vel rhodesiense, Human Strain, Nyasaland, and Trypanosoma brucei, Zululand Strain, 1913.

Experiment 1900.

In this experiment the trypanolytic action of serum of a dog immune to both *T. brucei vel rhodesiense*, Nyasaland (Naturally-Infected Dog Strain), and *T. brucei vel rhodesiense*, Nyasaland (Wild-Fly Strain), was tested on *T. brucei vel rhodesiense*, Nyasaland (Human Strain), and *T. brucei*, Zululand Strain.

This serum was shown to have a definite trypanolytic action upon *T. brucei*, Zululand Strain, and a still more marked action on *T. brucei vel rhodesiense*, Nyasaland (Human Strain), whereas normal human serum had little or no trypanolytic action on *T. brucei*, Zululand Strain.

The details of this experiment are as follows:—

12th February, 1913.—Five volumes of the serum of Dog 1253C immune to *T. brucei vel rhodesiense* (Wild-Fly Strain) and also to the "Naturally-Infected Dog Strain," were mixed with one volume of the blood of Rat 1828 containing *T. brucei*, Zululand Strain, and allowed to remain in contact for four hours at room temperature—24° C.

Result.—One large clump of degenerated trypanosomes seen, and several smaller clumps.

12th February, 1913.—Five volumes of the serum of the same dog were mixed with one volume of the blood of Rat 1817 containing *T. brucei vel rhodesiense* (Human Strain) and allowed to remain in contact for four hours at room temperature.

Result.—Very numerous clumps of degenerated trypanosomes; some attachment and phagocytosis.

12th February, 1913.—Five volumes normal human serum were mixed with one volume of blood of Rat 1828 containing *T. brucei*, Zululand Strain.

Result.—Negative, or very slight reaction.

The Trypanolytic action of Immune Serum on T. brucei vel rhodesiense, Human Strain, Nyasaland, and T. brucei, Zululand Strain, 1913.

Experiments 2106 and 2114.

* In these experiments the trypanolytic action was tested with the Zululand Strain and the Human Strain, Nyasaland. Immune guinea-pigs' serum was used with blood from infected rats.

The trypanolytic action of the Human Strain immune serum on the Zululand Strain was found to be markedly less than that of the Human Strain serum on the Human Strain trypanosome and the Zululand Strain serum on the Zululand trypanosome. But the Zululand serum gave a moderate to strong reaction with the Human Strain.

Conclusions.

It must be confessed that very little can be concluded from these serum experiments. They are too few in number, and are only given here to act as a slight basis for future work. The evidence supplied by these few experiments is in favour of *T. brucei*, Zululand Strain, and the Wild *G. morsitans* Strain, Nyasaland, belonging to the same species, and *T. brucei vel rhodesiense*, Human Strain, Nyasaland, belonging to a different species. The want of reaction to human serum as shown by the Human Strain may, however, be accounted for by its sojourn in human blood and subsequent acclimatisation.

XXXIV.—EXPERIMENTS ON IMMUNITY. *TRYPANOSOMA BRUCEI VEL RHODESIENSE*. THE NATURALLY-INFECTED DOG STRAIN.*

The following experiments were undertaken to find out whether the Naturally-Infected Dog Strain of the Trypanosome causing Disease in Man in Nyasaland would protect against the other strains. These different strains have been described in previous papers as the Human, the Wild Game, the Wild *Glossina morsitans*, Zululand, 1913, etc., and here they will be known by the same names. Human will, therefore, mean a strain of this species of trypanosome coming from man, Wild *Glossina morsitans* from a tsetse fly, and so on.

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 219.

These immunity experiments were necessarily one-sided, as it was, with three exceptions, only animals which had recovered from the weaker Naturally-Infected Dog Strain which were available.

There are practically no recoveries from the Human and other strains. A goat, monkey, and dog, apparently recovered from the Wild *Glossina morsitans* Strain, are included.

There are, therefore, no completed cross-inoculation experiments—or at least only one unsatisfactory one, Experiment 17—as would have been carried out if material had been forthcoming.

It will be seen from the following experiments that the Naturally-Infected Dog Strain failed to immunise animals against the Human, Wild *Glossina morsitans*, and Zululand, 1913, Strains; but it is not known whether these strains, on the other hand, would have immunised animals against the Naturally-Infected Dog Strain or not.

Experiment 1, Dog 459, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 April 20	459	From dog 317	Nat.-inf. dog 48	19	R
Nov. 22	459	From guinea-pig 1333	Human	4	59

Remarks.—Dog 459, which had recovered from Naturally-Infected Dog Strain 48, when inoculated with a Human Strain died in 59 days.

Conclusion.—The Naturally-Infected Dog Strain does not protect against the Human Strain IV., Chipochola.

Experiment 2, Monkey 1792, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1913 Jan. 22	1792	From rat 1741	Nat.-inf. dog 48	5	R
June 24	1792	" 2167	" 2033	—	—
July 12	1792	" 2235	Human	9	61

Remarks.—Monkey 1792, which had recovered from Naturally-Infected Dog Strain 48, and proved immune to Naturally-Infected Dog Strain 2033, succumbs to the Human Strain.

Conclusion.—The Naturally-Infected Dog Strain does not protect against the Human Strain, Yoram.

Experiment 3, Monkey 1793, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1913					
Jan. 22	1793	From rat 1741	Nat.-inf. dog 48	5	R
June 24	1793	" 2167	" 2033	—	—
July 12	1793	" 2235	Human	5	9

Remarks.—Monkey 1793, which had recovered from Naturally-Infected Dog Strain 48, and proved immune to Naturally-Infected Dog Strain 2033, succumbs in nine days to the Human Strain.

Conclusion.—The Naturally-Infected Dog Strain does not protect against the Human Strain, Yorumu.

Experiment 4, Monkey 2164, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1913					
May 14	2164	From rat 2091	Nat.-inf. dog 2033	—	—
June 14	2164	From dog 2157	" 2033	—	—
July 31	2164	From rat 2285	" 48	—	—
Dec. 17	2164	" 2437	Human	7	Alive 1.3.14

Remarks.—Monkey 2164, which was proved to be immune against the Naturally-Infected Dog Strains 2033 and 48, reacts readily to the Human Strain.

Conclusion.—Immunity to the Naturally-Infected Dog Strain does not imply immunity to the Human Strain, Dongolosi.

Experiment 5, Dog 690, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912					
July 17	690	Naturally-infected	Nat.-inf. dog 690	?	R
Nov. 22	690	From rat 1492	" 48	—	—
Dec. 20	690	From dog 1675	Wild <i>G. morsitans</i>	10	43

Remarks.—Dog 690, which had recovered from Naturally-Infected Dog Strain 690, and proved immune to Naturally-Infected Dog Strain 48, succumbs in 43 days to the Wild *G. morsitans* Strain.

Conclusion.—The Naturally-Infected Dog Strain does not protect against the Wild *G. morsitans* Strain.

Experiment 6, Dog 1530, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 Oct. 29	1530	From rat 1491	Nat.-inf. dog 48	16	R
1913 Mar. 21	1530	" 1991	" 48	—	—
April 11	1530	From guinea-pig 2034	Wild <i>G. morsitans</i>	10	23

Remarks.—Dog 1530, after having recovered from Naturally-Infected Dog Strain 48, and shown to be immune on re-injection, readily succumbs to one injection of the Wild *G. morsitans* Strain.

Conclusion.—The Naturally-Infected Dog Strain does not protect against the Wild *G. morsitans* Strain.

Experiment 7, Monkey 1534, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 Oct. 29	1534	From rat 1491	Nat.-inf. dog 48	6	R
1913 Mar. 21	1534	" 1991	" 48	—	—
April 11	1534	" 2020	Wild <i>G. morsitans</i>	6	17

Remarks.—Monkey 1534, having recovered from Naturally-Infected Dog Strain 48, and proved to be immune to the same strain, succumbs in 17 days to the Wild *G. morsitans* Strain.

Conclusion.—The Naturally-Infected Dog Strain does not protect against the Wild *G. morsitans* Strain.

Experiment 8, Monkey 1630, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 Nov. 22	1630	From monkey 1534	Nat.-inf. dog 48	10	R
1913 Mar. 21	1630	From rat 1991	48	—	—
April 11	1630	From guinea-pig 2034	Wild <i>G. morsitans</i>	13	85

Remarks.—Monkey 1630, having recovered from Naturally-Infected Dog Strain 48, and shown to be immune on re-injection, succumbs to the Wild *G. morsitans* Strain.

Conclusion.—The Naturally-Infected Dog Strain does not protect against the Wild *G. morsitans* Strain.

Experiment 9, Goat 427, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 April 20	427	From rat 392	Nat.-inf. dog 48	10	R
1913 Jan. 22	427	„ 1741	„ 48	—	—
Feb. 11	427	„ 1735	„ 48	—	—
Feb. 28	427	From dog 1906 and rat 1832	Zululand, 1913	38	54

Remarks.—Goat 427 has recovered from Naturally-Infected Dog Strain 48, and has shown no reaction to two re-injections, but when inoculated with *T. brucei*, Zululand, 1913, takes the disease and dies in 54 days.

Conclusion.—The Naturally-Infected Dog Strain does not protect against *T. brucei*, Zululand, 1913.

Experiment 10, Goat 432, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 April 20	432	From rat 392	Nat.-inf. dog 48	26	R
1913 Jan. 22	432	" 1741	" 48	—	—
Feb. 11	432	" 1735	" 48	—	—
Feb. 28	432	From dog 1906 and rat 1832	Zululand, 1913	31	143

Remarks.—Goat 432 has recovered from Naturally-Infected Dog Strain 48, but succumbs to *T. brucei*, Zululand, 1913.

Conclusion.—The Naturally-Infected Dog Strain does not protect against *T. brucei*, Zululand, 1913.

Experiment 11, Sheep 456, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 April 20	456	From rat 392	Nat.-inf. dog 48	5	R
1913 Mar. 21	456	" 1991	" 48	—	—
1914 Jan. 7	456	" 2470	Zululand, 1913	10	Alive 1.3.14

Remarks.—Sheep 456 has recovered from Naturally-Infected Dog Strain 48, but reacts when exposed to the virus of *T. brucei*, Zululand, 1913.

Conclusion.—The Naturally-Infected Dog Strain does not protect against *T. brucei*, Zululand, 1913.

Experiment 12, Dog 1253, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 Sept. 6	1253	From rat 1218	Nat.-inf. dog 48	6	R
1913 Jan. 4	1253	" 1570	" 48	5	R
Jan. 17	1253	" 1734	" 48	—	—
Feb. 28	1253	From dog 1906 and rat 1832	Zululand, 1913	6	48

Remarks.—Dog 1253 has recovered from Naturally-Infected Dog Strain 48, but succumbs to *T. brucei*, Zululand, 1913.

Conclusion.—The Naturally-Infected Dog Strain does not protect against *T. brucei*, Zululand, 1913.

Experiment 13, Monkey 1794, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1913 Jan. 22	1794	From rat 1741	Nat.-inf. dog 48	—	—
Feb. 28	1794	" 1945	" 48	—	—
May 23	1794	From monkey 2131	" 48	—	—
June 11	1794	" 2184	" 48	—	—
June 24	1794	From guinea-pig	Zululand, 1913	9	42

Remarks.—Monkey 1794 has been proved to be immune to Naturally-Infected Dog Strain 48, but when exposed to the Zululand Strain succumbs.

Conclusion.—Immunity to the Naturally-Infected Dog Strain does not imply immunity to *T. brucei*, Zululand, 1913.

Experiment 14, Monkey 1798, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1913 Jan. 22	1798	From monkey 1630	Nat.-inf. dog 48	—	—
Feb. 28	1798	From rat 1945	" 48	—	—
May 22	1798	From monkey 2131	" 48	—	—
June 11	1798	" 2184	" 48	—	—
June 24	1798	From guinea-pig 2225	Zululand, 1913	9	15

Remarks.—Monkey 1798 has been proved to be immune to Naturally-Infected Dog Strain 48, but succumbs to *T. brucei*, Zululand, 1913.

Conclusion.—The Naturally-Infected Dog Strain does not protect against *T. brucei*, Zululand, 1913.

Experiment 15, Monkey 2161, Naturally-Infected Dog Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1913 May 14	2161	From rat 2091	Nat.-inf. dog 2033	—	—
June 14	2161	From dog 2157	" 2033	—	—
July 31	2161	From rat 2285	" 48	—	—
Dec. 17	2161	" 2451	Zululand, 1913	7	Alive 1.3.14

Remarks.—Monkey 2161, proved to be immune to Naturally-Infected Dog Strains 2033 and 48, shows no immunity to *T. brucei*, Zululand, 1913.

Experiment 16, Goat 639, Wild *Glossina morsitans* Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 June 12	639	From rat 543	Wild <i>G. morsitans</i>	12	R
1913 Jan. 22	639	" 1741	Nat.-inf. dog 48	—	—
Feb. 11	639	" 1735	" 48	—	—
Feb. 28	639	From dog 1906 and rat 1832	Zululand, 1913	10	48

Remarks.—Goat 639 has recovered from the Wild *G. morsitans* Strain, has shown no reaction when inoculated with Naturally-Infected Dog Strain 48, but is killed in 48 days by *T. brucei*, Zululand, 1913.

Conclusion.—The Wild *G. morsitans* Strain, combined with the Naturally-Infected Dog Strain, has no protective power against *T. brucei*, Zululand, 1913.

Experiment 17, Monkey 970, Wild *Glossina morsitans* Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912 July 24	970	From rat 658	Wild <i>G. morsitans</i>	8	R
1913 Jan. 2	970	" 1664	"	—	—
Jan. 17	970	" 1740	"	—	—
Feb. 4	970	" 1814	Nat.-inf. dog 48	9	R
Feb. 28	970	" 1945	" 48	—	—
Mar. 15	970	From guinea-pig 1657	Human	9	71

Remarks.—Monkey 970, after recovering from the Wild *G. morsitans* Strain, shows a reaction of Naturally-Infected Dog Strain 48, and finally succumbs to a Human Strain.

Conclusion.—The Wild *G. morsitans* Strain does not protect against the Naturally-Infected Dog Strain, nor does the combination of the two against the Human Strain V., Chibibi.

Experiment 18, Dog 602, Wild *Glossina morsitans* Strain.

Date.	Expt.	Source of virus.	Strain of trypanosome.	Period of incubation, in days.	Duration of disease, in days, or recovery.
1912					
May 31	602	Wild flies	Wild <i>G. morsitans</i>	5	R
Nov. 22	602	From guinea-pig 1333	Human	6	28

Remarks.—Dog 602, which had recovered from the Wild *G. morsitans* Strain, when inoculated with a Human Strain died in 28 days.

Conclusion.—The Wild *G. morsitans* Strain does not protect against the Human Strain IV., Chipochola.

Conclusions.

1. The Naturally-Infected Dog Strain does not protect animals from the Human, Wild *Glossina morsitans*, and Zululand, 1913, Strains.

2. The Wild *Glossina morsitans* Strain and the Naturally-Infected Dog Strain do not protect animals from the Human or the Zululand, 1913, Strain.

3. The Wild *Glossina morsitans* Strain does not protect against the Human Strain.

4. In spite of the damaging evidence of these experiments, the Commission still hold the opinion that the Naturally-Infected Dog Strain is a weak strain of the Trypanosome causing Disease in Man in Nyasaland, *Trypanosoma brucei vel rhodesiense*.

D.—TRYPANOSOME DISEASES OF DOMESTIC ANIMALS IN NYASALAND.

XXXV.—*Trypanosoma Pecorum*.

INTRODUCTION.

This species was described in the Reports of the Sleeping Sickness Commission, No. XI., p. 128, as occurring in Uganda.

To this type belong *Trypanosoma dimorphon* (Laveran and Mesnil) and *Trypanosoma congolense* (Brodin). It is still a debated question as to whether *Trypanosoma pecorum*, the cattle trypanosome of Central and Eastern Africa, is or is not the same species as *Trypanosoma dimorphon* and *Trypanosoma congolense* of the West Coast. Be this as it may, there can be little doubt that the three are very closely related.

The Morphology of Trypanosoma pecorum.

This paper has already been published in the "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 1, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 78.

Three strains were investigated, viz., a cattle, wild game, and wild *Glossina morsitans* strain, and the conclusion arrived at is that it is identical in regard to morphology with the species found and described in Uganda.

The Susceptibility of Animals to Trypanosoma pecorum.

This paper was published as part of the preceding one in the "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 15, and reprinted in the Reports of the Sleeping Sickness Commission, vol. XV., p. 89.

The susceptibility of animals to the same three strains was inquired into, and it was concluded that:—

It is an important disease of domestic animals in Nyasaland, being destructive to donkeys, oxen, goats, pigs, and dogs.

Its carrier was found to be, in the district near Kasu, *G. morsitans*, about 2 per cent. of the local wild flies being naturally-infected with the trypanosome.

The reservoir was wild game inhabiting the "fly-country," and 14.4 per cent. were found to be infected with *T. pecorum*.

A recommendation was also made that if infected animals are found in a herd, they should be destroyed or segregated, as there may be a danger of biting flies other than the tsetse spreading the disease in the herd by mechanical transmission.

*The Development of Trypanosoma pecorum in Glossina morsitans.**

This trypanosome belongs to the group in which the development takes place first in the gut, then passes forward into the labial cavity of the proboscis, and finally reaches the hypopharynx, where the trypanosomes revert to the original "blood forms" and become infective. There is no infection of the salivary glands.

Seven experiments were carried out with laboratory-bred flies. Five were positive and two negative.

Table CXXX.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Mean Temperature.
1912						
May 16	546	22	+	4	53	69°F. (20.5°C.)
July 2	524	20	+	2	37	65°F. (18.3°C.)
1913						
Jan. 3	1732	60	—	0	—	84°F. (28.8°C.)
Jan. 7	1737	40	+	3	19	"
Feb. 10	1853	25	+	5	24	"
Feb. 24	1950	33	+	6	21	"
April 29	2115	40	—	4	—	"

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 33.

Two hundred and forty flies were used and 24 infected flies found ---10 per cent. The first two experiments were carried out at the ordinary temperature of the laboratory; in the others the flies were kept in the incubator.

Details of the Five Positive Experiments.

The following tables give the principal details in the carrying out of the five positive experiments. They were all carried out with laboratory-bred flies.

Table CXXXI.

Expt.	Day of expt.	Procedure.	Remarks.
546	1-3	22 flies fed on <i>T. pecorum</i> —infected dog	Goat 559 became infected on the 60th day; dog 880 on the 82nd day. All flies dissected; four found infected.
	4	Starved	
	5-62	Fed on clean goat 559	
	63	Starved	
	64-82	Fed on clean dog 880	
524	1-5	20 flies fed on <i>T. pecorum</i> —infected rat	Trypanosomes first appeared in blood of dog 541 on the 44th day. All flies dissected; two found infected.
	6	Starved	
	7-44	Fed on clean dog 541	
1737	1-3	40 flies fed on <i>T. pecorum</i> —infected dog	Trypanosomes first appeared in blood of dog 1750 on the 26th day. All flies dissected; three found infected.
	4	Starved	
	5-27	Fed on clean dog 1750	
1853	1-3	25 flies fed on <i>T. pecorum</i> —infected dog	Trypanosomes first appeared in blood of goat 1903 on the 31st day. 25 flies dissected; five found infected.
	4	Starved	
	5-25	Fed on clean goat 1903	
1950	1-8	33 flies fed on <i>T. pecorum</i> —infected goat	Trypanosomes first appeared in blood of dog 1973 on the 28th day. All flies dissected; six found infected.
	9	Starved	
	10-29	Fed on clean dog 1973	

It would appear from these five positive experiments that a period of from 19 to 53 days may elapse before the cycle of development of *Trypanosoma pecorum* in *Glossina morsitans* is complete, and the fly becomes infective.

Details of the Two Negative Experiments.

The following table shows the method of procedure in carrying out the two negative experiments.

Table CXXXII.

Expt.	Day of expt.	Procedure.	Remarks.
1732	1—2 3 4—39	60 flies fed on <i>T. pecorum</i> —infected dog Starved Fed on clean dog 1736 (Experiment stopped)	Dog 1736 never showed trypanosomes. 25 flies remained alive; used for another experiment. Only 12 flies dissected, all negative.
2115	1—4 5—6 7—8	40 flies fed on <i>T. pecorum</i> —infected rat. Starved Fed on clean monkey 2066 (Experiment stopped)	Monkey 2066 never showed trypanosomes. All flies dissected; four found infected. Experiment stopped on account of death of most of flies.

Result of the Dissection of the Infected Flies.

The following table gives the result of the dissection of the infected flies found in the positive experiments. The second column gives the number of days between the first infected feed of the fly and its death and dissection.

Table CXXXIII.—Laboratory-bred Flies. Positive Experiments.

Expt.	Time, days.	Proboscis.		Proventriculus.	Crop.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands
546	30	—	—	—	—	+	+	+	—
"	64	—	—	—	—	++	++	++	—
"	84	—	—	—	—	++	++	++	—
"	84	++	—	++	++	++	++	++	—
524	27	—	—	—	—	—	++	++	—
"	55	+	—	+	—	++	++	++	—
		Labial cavity.	Hypo-pharynx.						
1737	27	++	++	++	++	++	++	++	—
"	28	+	—	+	—	++	++	++	—
"	30	++	++	++	—	++	++	++	—
1853	17	++	++	++	—	++	++	++	—
"	22	—	—	—	—	+	++	+	—
"	23	+	—	—	—	++	++	++	—
"	25	++	++	+	—	++	++	++	—
"	26	++	++	+	—	++	++	++	—
1950	17	—	—	+	—	++	++	++	—
"	19	++	++	—	—	++	++	++	—
"	24	—	—	—	—	—	+	—	—
"	26	++	++	+	—	++	++	++	—
"	31	++	++	+	—	++	++	++	—
"	31	++	++	++	—	++	++	++	—

In Experiments 546 and 524 there was no special examination of the hypopharynx; it is included in the general term "Proboscis." It was only after the importance of the hypopharynx became evident that an examination of these separate parts of the proboscis was made.

In Experiment 546 only one infective fly was found. In Experiment 524 two infected flies were found; in one of these the development was incomplete, in the other, complete. In 1737 two flies were infective; in 1853, three; and in 1950, four. In not a single fly was any invasion of the salivary glands noted.

The following table gives the result of the dissection of the infected flies in the negative experiments.

Table CXXXIV.—Laboratory-bred Flies. Negative Experiments.

Expt.	Time, days.	Proboscis.		Proventriculus.	Crop.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
		Labial cavity.	Hypopharynx.						
2115	9						+		
"	9						++		
"	9	+		+		+	++	++	
"	11			+		+	++	+	

In the negative Experiment 1732, all the flies were found to be negative.

In Experiment 2115, four infected flies were found, but in none of these had the development reached the hypopharynx; none of them were infective.

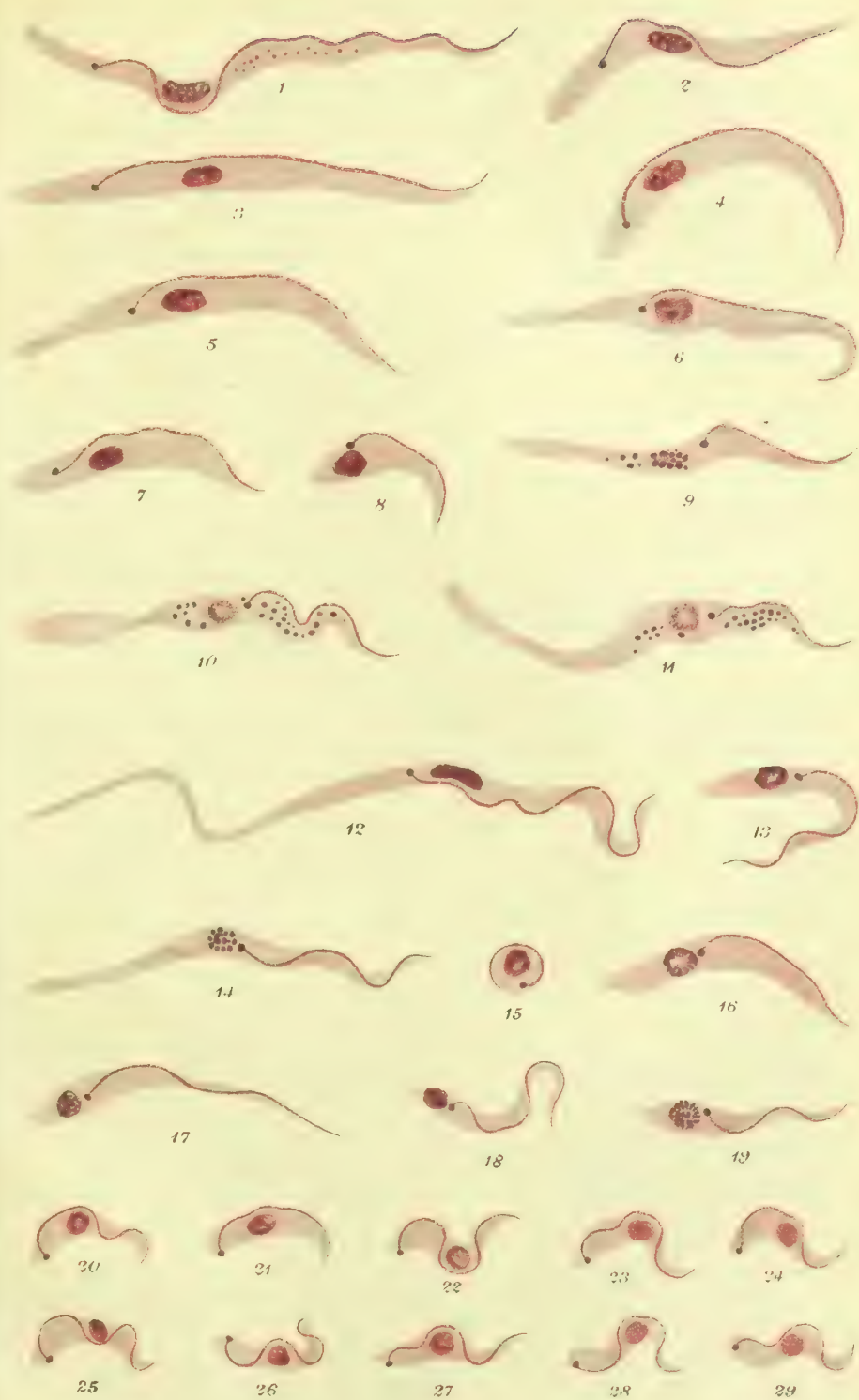
From a consideration of these tables it will be seen that *Trypanosoma pecorum* belongs to the same group as *Trypanosoma simia* as regards its development in *Glossina morsitans*. This development takes place at first in the intestine, then passes forward into the labial cavity, and finally invades the hypopharynx, and there is completed.

The type of Trypanosomes found in the Infected Flies.

Plate 10 represents the developmental forms of *Trypanosoma pecorum* in *Glossina morsitans*. In regard to the forms found in the intestine, it may be said that these are indistinguishable from the developmental forms of other pathogenic trypanosomes, and what was written in regard to *Trypanosoma simia** is equally applicable to *Trypanosoma pecorum*.

Figs. 1 and 2 are forms from the proventriculus, and represent the dominant intestinal trypanosome forms passing forward to the labial cavity.

* "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 65; and Reports of the Sleeping Sickness Commission, No. XV., p. 131.



Trypanosoma pecorum
Development in *Glossina morsitans*

Figs. 3-8 represent early forms found in the labial cavity. These were seen adhering singly by their flagella to the labrum.

Figs. 9-11 are the ordinary forms found clinging by their flagellar ends to the labrum. It will be seen that they have assumed the crithidial stage, a stage which seems to be a *sine qua non* in the final stages of the cycle of development of all the pathogenic trypanosomes, the interpretation of which is still obscure.

Figs. 12-19 are various forms other than "blood forms" which have been squeezed out of the proboscis of a living infective fly. Fig. 15 appears to be encysted.

Figs. 20-29 are "blood forms" from the hypopharynx of dead infective flies, and also from living flies induced to salivate on a cover-glass. They represent the final stage in the cycle of development, and are the only infective forms.

Conclusions.

1. That *Trypanosoma pecorum* is capable of passing through a cycle of development in *Glossina morsitans*, the flies becoming infective some 20 days after feeding on an infected animal.

2. That *Trypanosoma pecorum* belongs to the same group as *Trypanosoma simia*, the development taking place at first in the gut and afterwards passing forward into the labial cavity, and finally into the hypopharynx.

3. That the final stage of the development only occurs in the hypopharynx, where the trypanosomes revert to the "blood form" and become capable of setting up infection if injected under the skin of healthy animals.

The Development of *Trypanosomes pecorum* in *Glossina brevipalpis*.*

(a) Feeding wild *Glossina brevipalpis* first on animals infected with *Trypanosoma pecorum* and then on healthy animals, to ascertain if this species of trypanosome passes through a cycle of development in this species of tsetse fly.

Table CXXXV.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No of flies dissected.	No. of infected flies found	No. of days before flies became infected.
1912						
May 28	2190	80	+	17	6	29
June 2	2207	50	—	16	0	—

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 29.

One hundred and thirty wild *Glossina brevipalpis* were used in two experiments—one positive, one negative. Only 33 flies were dissected; six found infected.

(b) *Details of the Two Experiments.*

Table CXXXVI.

Expt.	Day of expt.	Procedure.	Remarks.
2190	1 3	Flies fed on infected goat 2186	Eighty flies used. Six infected flies found, only 17 dissected. Goat 2202 showed trypanosome on the 36th day.
	4—5	Starved	
	6—36	Fed on clean goat 2202	
2207	1—6	Flies fed on infected goat 2126	Fifty flies used. Only 16 flies dissected, all negative.
	7	Starved	
	8—46	Fed on clean goat 2210	

(c) *Result of the Dissection of the Infected Flies.*

Table CXXXVII.

Expt.	Time, days.	Proboscis.		Alimentary tract.	Salivary glands.
		Labial cavity.	Hypopharynx.		
2190	23	—	—	+	—
"	23	+	++	+	—
"	36	—	—	+	—
"	36	+	++	+	—
"	36	+	++	+	—
"	36	+	++	+	—

In Experiment 2190, six infected flies were found. In four of these the hypopharynx was blocked with small "blood forms" of *Trypanosoma pecorum*. Taking into consideration the time which elapsed between the feeding on the infected goat and the appearance of an infective fly in the cage—23 days—and, further, the number of flies found infected with *Trypanosoma pecorum*—4 in 33—it must be admitted that a development of *Trypanosoma pecorum* has taken place in *Glossina brevipalpis*.

Conclusions.

Glossina brevipalpis is capable of acting as a carrier of *Trypanosoma pecorum*.

XXXVI.—*TRYPANOSOMA SIMIÆ*, SP. NOV.*The Morphology of Trypanosoma Simiæ.*

An account of the morphology of this new species of trypanosome has previously been published in the "Proceedings" of the Royal Society, B, vol. 85, 1912, p. 477, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 11. A coloured plate showing *T. simiæ* from the monkey and the goat is also given.

T. simiæ is a well-defined species, easily separated by its morphology alone from the other trypanosomes which have been described as causing disease among domestic animals. It sets up a chronic disease in goats, but is chiefly remarkable for its rapidly fatal action on monkeys and the domestic pig. In Nyasaland it is carried by *G. morsitans*, and in the district round Kasu this tsetse fly is found to be heavily infected with this trypanosome. Up to the present time the only wild animal found infected with this trypanosome is the warthog (*Phacochoerus aethiopicus*).

The Susceptibility of Animals to Trypanosoma simiæ.

This paper has already appeared in the "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 48, and has been reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 121.

The conclusions arrived at are:—

1. *T. simiæ* belongs to the same group as *T. pecorum*, and, like the latter, is erratic in its action on animals.
2. *T. simiæ* affects goats, sheep, pigs, and monkeys. Oxen, antelope, dogs, rabbits, guinea-pigs, and rats are practically immune.

The Development of Trypanosoma simiæ in Glossina morsitans.

This paper was published in the "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 58, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 131.

It is an account of eight experiments on the development of *Trypanosoma simiæ* in laboratory-bred *Glossina morsitans*. In the introduction to the paper a general survey of the various modes of development which take place in the different groups of trypanosomes is given.

One coloured plate of developmental forms of *T. simiæ* from the proboscis and hypopharynx of *G. morsitans* is given; also two black and white drawings of the trypanosome as seen in the labrum, hypopharynx, and gut.

It was concluded:—

1. That *T. simiæ* can be transmitted from infected to healthy animals by the tsetse fly *G. morsitans*.

2. That *T. simiæ* multiplies in the intestines and in the labial cavity of the proboscis of the fly. Here only developmental forms are found, never infective forms.

3. That the *T. simiæ* growing in the alimentary canal has no specific characters by which it can be distinguished from other species of pathogenic trypanosomes found in the gut of tsetse flies.

4. That the final stage of the development takes place in the hypopharynx, wherein the infective form of the parasite, similar in shape to the trypanosome found in the blood of infected animals, is produced.

5. That the flies do not become infective until about 20 days after their first infected feed.

XXXVI.—*TRYPANOSOMA CAPRÆ* (KLEINE).

The Morphology of Trypanosoma capræ.

This paper was published in the "Proceedings" of the Royal Society, B, vol. 86, 1913, p. 278, and reprinted in the Reports of the Sleeping Sickness Commission, No. XV., p. 25.

One coloured plate of *T. capræ*, as found in the goat, sheep, ox, and waterbuck is given.

The following conclusions, which refer to the next paper also, are given:—

1. *T. capræ* belongs to the same group as *T. vivax* and *T. uniforme*, and affects the same animals—cattle, goats, and sheep, Monkeys, dogs, and the smaller laboratory animals are immune.

2. The carrier is *G. morsitans*.

3. The reservoir of the virus is the wild game living in the fly-country.

The Susceptibility of Animals to Trypanosoma capræ.

This paper was published and reprinted with the preceding one, of which it formed part.

Cattle, goats, sheep, monkeys, dogs, guinea-pigs, and rats were inoculated with *T. capræ* to test their susceptibility, and as in *T. vivax* and *T. uniforme*, the same animals were found to become infected, viz., cattle, goats, and sheep. Monkeys, dogs, and the smaller laboratory animals were found to be immune.

*The Development of Trypanosoma capræ in Glossina morsitans.**

Trypanosoma capræ belongs to the *Trypanosoma vivax* group, in which the development of the trypanosomes is restricted to the proboscis.

Six experiments were made with laboratory-bred flies. Five were positive and one negative.

Table CXXXVIII.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Mean temperature.
1912.						
April 16	444	12	+	1	16	71°F. (22 1°C).
June 3	617	33	-	0		65°F. (18 3°C).
" 3	1215	22	+	1	21	" "
1913.						
Jan. 18	1777	35	+	11	19	84°F. (28 8°C).
Jan. 22	1784	35	+	20	19	" "
April 1	2046	33	+	13	20	" "

One hundred and seventy laboratory-bred flies were used and 46 infected flies were found—27.1 per cent. The three first experiments were carried out at the ordinary temperature of the laboratory; in the last three the cages containing the flies were kept in an incubator. It is difficult to understand the difference in the number of infected flies found. In Experiments 444 and 1215 only 8 and 5 per cent., respectively, of the flies became infected, whereas in the three last experiments, an average of more than 40 per cent was found. The flies in the second group were kept, it is true, at a temperature similar to that which they would find in summer in the low-country, while the first three experiments were done in winter and at the ordinary temperature of the laboratory. This no doubt would explain the difference to some extent. Again, goats and sheep infected with *Trypanosoma capræ* are unsatisfactory animals to feed flies on. One day the trypanosomes are present in small numbers in the blood, the next day it may be impossible to find any; very seldom are they in any numbers. It is quite possible, then, that flies may feed on an infected goat or sheep without taking in a single trypanosome.

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, 1914, p. 92.

Details of the Six Experiments. Five Positive, One Negative.

The following table gives the principal details in carrying out the six experiments. Laboratory-bred flies were used in all.

Table CXXXIX.

Expt.	Day of expt.	Procedure.	Remarks.
444	1-4	12 flies fed on infected goat 339	Trypanosomes appeared in blood of goat 419 after 23 days. All flies dissected; one infected fly found. Goat 339 contained few trypanosomes in its blood.
	5-6	Starved	
	7-24	Fed on clean goat 419	
617	1-4	33 flies fed on infected sheep 347	Trypanosomes never appeared in blood of goat 628. All flies dissected; all negative. Sheep 347 was unsatisfactory; one day its blood contained a few trypanosomes, the next day none.
	5	Starved	
	6-63	Fed on clean goat 628	
1215	1-3	22 flies fed on infected goat 979	Trypanosomes appeared in blood of goat 1219 after 28 days. All flies dissected; one infected fly found.
	4	Starved	
	5-29	Fed on clean goat 1219	
1777	1-5	35 flies fed on infected goat 1746	Trypanosomes appeared in blood of goat 1803 after 26 days. All flies dissected; 11 infected flies found.
	6	Starved	
	7-27	Fed on clean goat 1803	
1784	1-4	35 flies fed on infected goat 1746	Trypanosomes appeared in blood of goat 1812 after 26 days. All flies dissected; 20 found infected.
	5	Starved	
	6-27	Fed on clean goat 1812	
2046	1-5	33 flies fed on infected goat 1912	Trypanosomes appeared in blood of goat 2102 after 27 days. All flies dissected; 13 found infected.
	6	Starved	
	7-18	Fed on clean monkey 2066	
	19-20	Starved	
	21-23	Fed on clean goat 2102	
	24-25	Starved	
	26-36	Fed on clean monkey 2066	

It would appear from the five positive experiments that an average period of 19 days elapses before the cycle of development of *Trypanosoma caprae* is complete in *Glossina morsitans* and the fly becomes infective.

Result of the Dissection of the Infected Flies.

Table CXL.—Laboratory-bred Flies. Positive Experiments.

[Expt.	Time, days.	Proboscis.		Provent- riculus.	Crop.	Fore- gut.	Mid- gut.	Hind- gut.	Salivary glands.
444	25	+		—	—	—	—	—	—
1215	32	+		—	—	—	—	—	—
1777	21	+		—	—	—	—	—	—
"	26	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
1784	19	+		—	—	—	—	—	—
"	21	+		—	—	—	—	—	—
"	23	+		—	—	—	—	—	—
"	24	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	29	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
"	30	+		—	—	—	—	—	—
		Labial cavity.	Hypo- pharynx.						
2046	23	+	+	—	—	—	—	—	—
"	23	+	—	—	—	—	—	—	—
"	24	+	++	—	—	—	—	—	—
"	26	++	++	—	—	—	—	—	—
"	28	+	+	—	—	—	—	—	—
"	28	+	+	—	—	—	—	—	—
"	29	++	++	—	—	—	—	—	—
"	29	+	++	—	—	—	—	—	—
"	29	+	++	—	—	—	—	—	—
"	29	+	++	—	—	—	—	—	—
"	29	++	++	—	—	—	—	—	—
"	29	++	+	—	—	—	—	—	—
"	30	+	++	—	—	—	—	—	—

It will be seen from the above table that it was not until the last experiment that the labial cavity and hypopharynx were examined

separately. In the previous experiments the presence or absence of trypanosomes in the proboscis as a whole was noted.

In the first two experiments, only a single infected fly was found in each. In Experiment 1777 eleven, and in 1784 as many as twenty were found.

In regard to the number of trypanosomes in the labial cavity, this may vary greatly. Sometimes the lumen of the tube will be seen to be densely crowded; at other times a single colony will be seen. For example, in Experiment 1777 the first infected fly, dissected on the twenty-first day, is noted to have had the lumen of the proboscis swarming with clusters of torpedo-shaped flagellates attached to the labrum by their flagellar ends, a few swimming free in the lumen. In the seventh infected fly, dissected on the thirtieth day, only three colonies, in the eighth one, and in the ninth two small colonies, are noted. In the same way the hypopharynx may contain few; at other times it is seen to be densely packed with swarms of actively moving trypanosomes. In unstained specimens the difference in size and shape between the trypanosomes in the labial cavity and those in the hypopharynx is quite manifest.

It may be stated here that, exceptionally, flagellates may be seen in the oesophagus, or that part of the alimentary tract anterior to the proventriculus. Among the 46 flies described above, this was noted twice. In the first instance they are reported as being very scanty, in the second as being active and in large numbers.

But from Table CXL. the broad fact stands out boldly—that in this species of trypanosome the development is confined to the labial cavity and hypopharynx, and does not take place in any other part of the fly.

The Type of Trypanosomes found in the Infected Flies.

No attempt has been made by the Commission to study the development of *Trypanosoma capræ* in *Glossina morsitans* in the earliest stages. This can only be done if a large number of laboratory-bred flies are available, and this was not the case at Kasu.

Plate 11 represents some of the developmental forms found in the labial cavity and hypopharynx of infected flies.

Fig. 1 represents a torpedo-shaped organism taken from a single cluster growing near the bulb on the 19th day after the first infected feed.

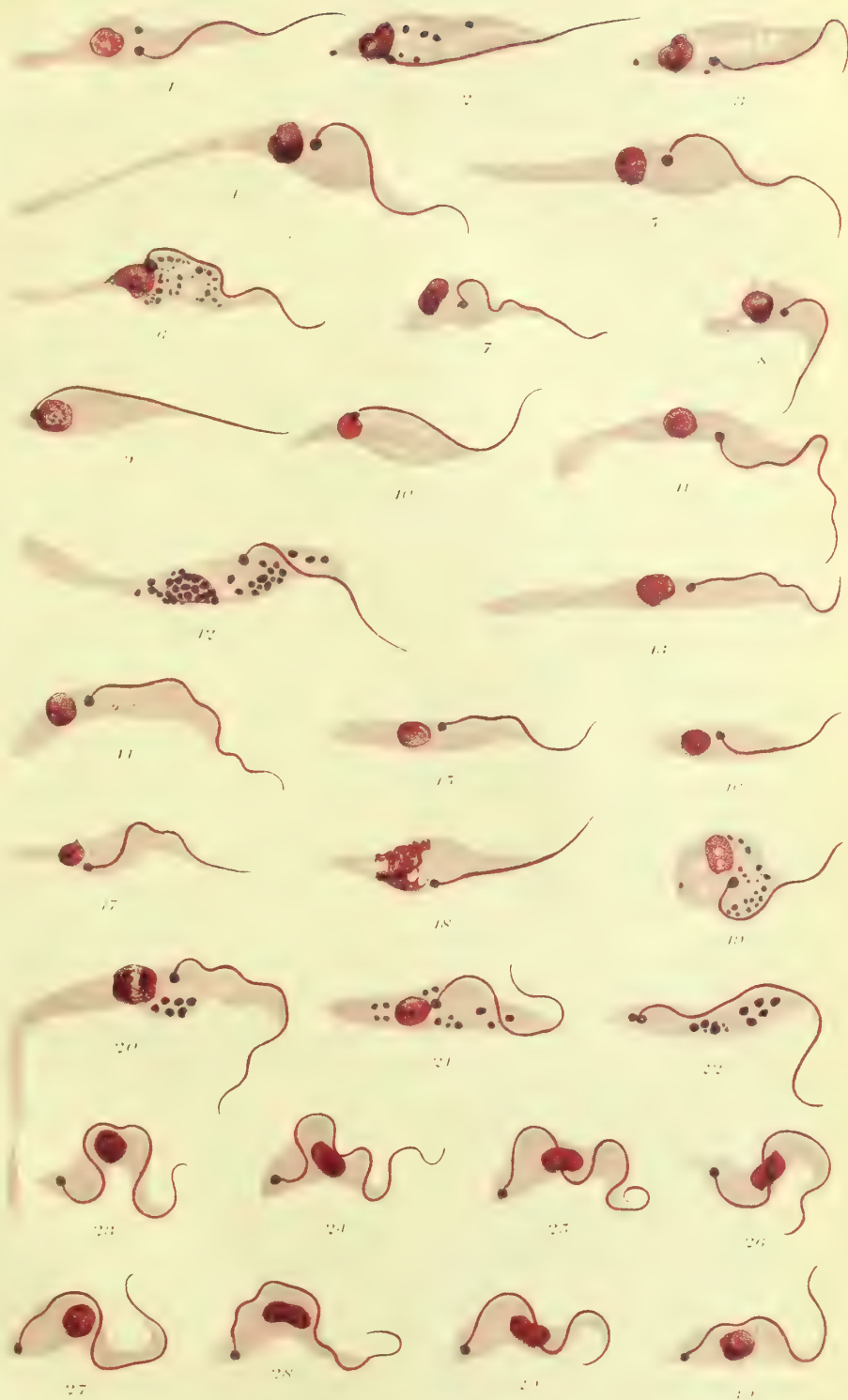
Figs. 2 and 3 are similar shaped flagellates, also form a single group growing near the bulb on the twenty-first day.

Figs. 4-10 are drawn from 24-day flies.

Figs. 11-19, 29 days. Fig. 19 has an encysted appearance.

Figs. 20-22, 30 days. It will be seen that most of the flagellates found in the labial cavity are crithidial in type. They are generally ribbon-shaped, with well defined nuclei and micronuclei and free flagella.

Figs. 23-30 are from the hypopharynx, and have been obtained, as a rule, by causing the fly to salivate on to a cover-glass. They represent the final stage in the cycle of development—the reversion to the infective or “blood form.” They are smaller than those found in the blood of the vertebrate host, but resemble them closely in every other way.



Trypanosoma capre
Development in *Glossina morsitans*

*The Development of Trypanosoma capræ in Glossina brevipalpis.**
Feeding Wild Glossina brevipalpis first on animals infected with Trypanosoma capræ and then on healthy animals, to ascertain if this species of trypanosome passes through a cycle of development in this species of tsetse fly.

Three experiments were carried out with wild flies. One was negative and two positive. On examining the positive experiments they were found to be *Trypanosoma pecorum* infections and not *Trypanosoma capræ*. The animals were probably infected by naturally-infected *Glossina brevipalpis*. No infection by *Trypanosoma capræ* took place, but one of the flies on dissection was shown to have an undoubted development of *Trypanosoma capræ* in the labial cavity and hypopharynx.

Table CXLI.

Date.	Expt.	No of flies used.	Experiment positive or negative.	No. of flies dissected.	No. of infected flies found.	No. of days before flies became infective.
1912						
April 11	2071	11	—	3	1	—
May 30	2199	60	+	25	1	20?
July 17	2277	50	+	0	0	24?

Details of the Three Experiments.

Table CXLII.

Expt.	Date of expt.	Procedure.	Remarks.
2071	1—8 9—10 11—12	Flies fed on infected goat 1912 Starved Fed on clean goat 2103	Eleven flies used. One infected fly found. Only three dissected.
2199	1—8 9 10—28 29—30 31—50	Flies fed on infected goat 1912 Starved Fed on clean goat 2212 Starved Fed on clean goat 2245	Sixty flies used. One infected fly found. 25 dissected. Goat 2212 showed trypanosomes on the 27th day. Goat 2245 negative.
2277	1—5 6—7 8—32 33—34 35—49	Flies fed on infected goat 2220 Starved Fed on clean goat 2287 Starved Fed on clean goat 2362	Fifty flies used. No flies dissected. Goat 2287 showed trypanosomes on the 31st day, and goat 2362 on the 49th day.

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 88, p. 30, 1914.

Result of the Dissection of the Two Infected Flies.

Table CXLIII.

Expt.	Time, days.	Proboscis.		Alimentary tract.	Salivary glands.
		Labial cavity.	Hypo- pharynx.		
2071	9	+	++	—	—
2199	45	++	—	+++	—

In Experiment 2071 there is evidently a development of *Trypanosoma capræ* in the fly found infected. The intestine and salivary glands are free, whereas the hypopharynx is crammed with numerous short trypanosomes of the *Trypanosoma capræ* type. In the labial cavity one large colony of large flagellates of a crithidial type was seen.

In Experiment 2199 the intestine and labial cavity of the infected fly were found to have a heavy infection of trypanosomes. As the intestine was also involved, this is probably a natural infection of the fly with *Trypanosoma pecorum*. The animal the flies were fed on was found to be suffering from a *Trypanosoma pecorum* infection.

In the third experiment—2277—none of the flies were dissected, but as the animal the flies were fed on became infected, as in the last experiment, with *Trypanosoma pecorum* and not with *Trypanosoma capræ*, it is probable that no complete development of the latter had taken place.

Conclusion.

Glossina brevipalpis is capable of acting as a carrier of *Trypanosoma capræ*.

General Conclusion.

Glossina brevipalpis is capable of acting as a carrier of *Trypanosoma brucei* vel *rhodesiense*, the trypanosome causing disease in man in Nyasaland, *Trypanosoma brucei*, Zululand Strain, 1913, *Trypanosoma pecorum*, and *Trypanosoma capræ*.

E.—DESCRIPTION OF THREE STRAINS OF *TRYPANOSOMA GAMBIENSE* FROM LAKE TANGANYIKA.XXXVII.—THE MORPHOLOGY OF *TRYPANOSOMA GAMBIENSE*, TANGANYIKA, STRAINS I., II., AND III.

At the request of the Commission, Dr. Kleine, Director of the Sleeping Sickness Commission, German East Africa, kindly sent three strains of *Trypanosoma gambiense* from Lake Tanganyika, in

order that a comparison on the spot might be made between this species and *Trypanosoma brucei* vel *rhodesiense*, the Trypanosome causing Disease in Man in Nyasaland.

The strains were inoculated into monkeys from natives suffering from Uganda sleeping sickness. These monkeys arrived safely at Kasu camp in February, 1913, and were at once sub-inoculated into a series of animals.

From the beginning great difficulty was experienced in keeping the strains alive in animals. Strain II. only showed in one rat. It was often only by repeated inoculations into the same animal that infection took place and Strains I. and III. were kept going.

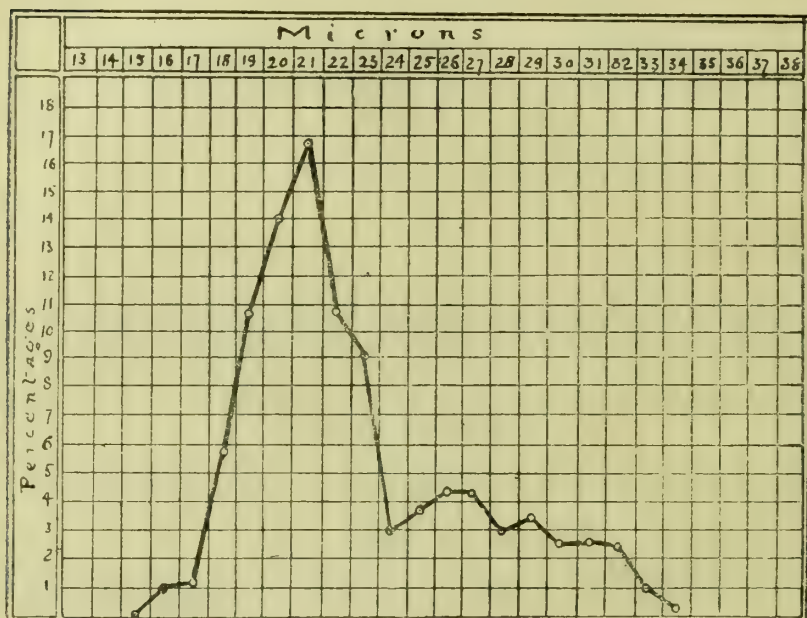
Morphology of Trypanosoma gambiense, Tanganyika, Strain I.

Length.—The following table gives the length of the trypanosome of Strain I., as found in the white rat—500 trypanosomes in all.

Table CXLIV.—Measurements of the Length of *T. gambiense*, Tanganyika, Strain I.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Mar. 28	1866	Rat	Osmic acid	Giemsa	24.6	33.0	17.0
" 28	1866	"	"	"	25.6	32.0	20.0
" 28	1866	"	"	"	21.9	33.0	18.0
April 5	1866	"	"	"	20.5	26.0	18.0
" 5	1866	"	"	"	22.6	32.0	19.0
" 5	1866	"	"	"	23.3	31.0	19.0
" 6	1866	"	"	"	23.6	32.0	20.0
" 6	1866	"	"	"	22.0	29.0	18.0
" 6	1866	"	"	"	22.5	30.0	19.0
" 7	1866	"	"	"	22.7	29.0	18.0
" 7	1866	"	"	"	22.4	30.0	19.0
" 7	1866	"	"	"	23.1	33.0	19.0
" 14	1866	"	"	"	23.0	32.0	19.0
" 14	1866	"	"	"	22.5	32.0	19.0
" 14	1866	"	"	"	21.8	31.0	18.0
" 15	1866	"	"	"	24.1	31.0	17.0
" 15	1866	"	"	"	24.1	31.0	18.0
" 15	1866	"	"	"	23.8	29.0	18.0
" 16	1866	"	"	"	26.1	33.0	20.0
" 16	1666	"	"	"	23.6	34.0	20.0
" 16	1866	"	"	"	23.3	31.0	19.0
" 17	1866	"	"	"	20.6	32.0	15.0
" 17	1866	"	"	"	20.5	27.0	17.0
" 17	1866	"	"	"	19.0	24.0	16.0
" 22	1866	"	"	"	20.5	32.0	18.0
					22.7	34.0	15.0

CHART 33.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *Trypanosoma gambiense*, Tanganyika, Strain I., taken on 9 consecutive days from Rat 1866.



Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all.

Table CLXV.—Measurements of the Breadth of *Trypanosoma gambiense*, Tanganyika, Strain I., measured across the widest part, including the undulating membrane.

Expt. No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
1866	Rat	500	2.86	4.50	1.25

Posterior-nuclear forms.—0 per cent.

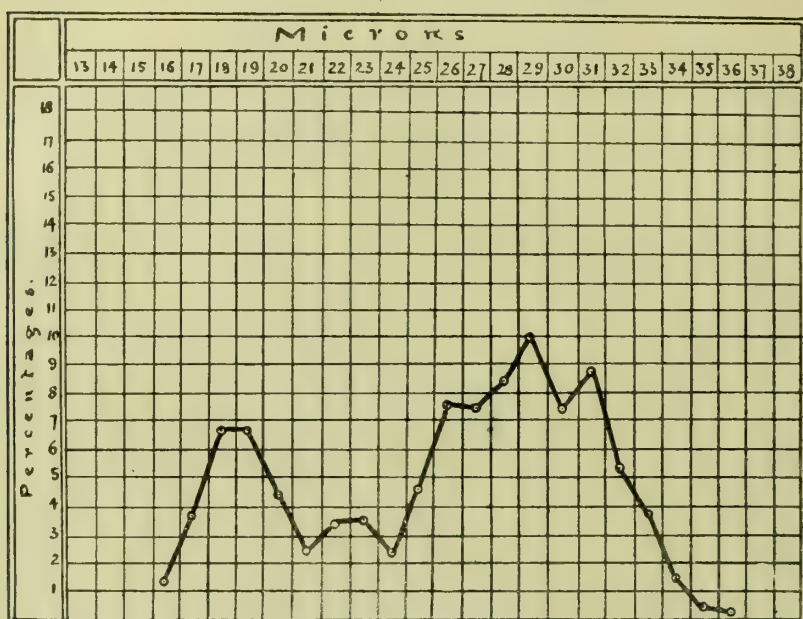
Morphology of Trypanosoma gambiense, Tanganyika. Strain II.

Length.—The following table gives the length of the trypanosome of Strain II., as found in the white rat—500 trypanosomes in all.

Table CXLVI.—Measurements of the Length of *T. gambiense*, Tanganyika, Strain II.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Feb. 6	1816	Rat	Osmic acid	Giemsa	29.9	34.0	26.0
" 6	1816	"	"	"	26.9	31.0	18.0
" 6	1816	"	"	"	28.8	32.0	19.0
" 7	1816	"	"	"	28.0	33.0	18.0
" 7	1816	"	"	"	27.7	34.0	19.0
" 7	1816	"	"	"	27.8	33.0	22.0
" 8	1816	"	"	"	25.9	35.0	17.0
" 8	1816	"	"	"	27.3	35.0	17.0
" 8	1816	"	"	"	28.6	36.0	19.0
" 13	1816	"	"	"	27.8	34.0	19.0
" 13	1816	"	"	"	29.1	33.0	24.0
" 13	1816	"	"	"	28.2	31.0	19.0
" 14	1816	"	"	"	25.3	32.0	17.0
" 14	1816	"	"	"	24.4	30.0	16.0
" 14	1816	"	"	"	27.4	33.0	18.0
" 15	1816	"	"	"	23.3	31.0	16.0
" 15	1816	"	"	"	22.8	30.0	17.0
" 15	1816	"	"	"	26.1	32.0	18.0
" 16	1816	"	"	"	24.0	33.0	17.0
" 16	1816	"	"	"	22.9	33.0	16.0
" 16	1816	"	"	"	24.9	32.0	19.0
" 17	1816	"	"	"	22.4	33.0	16.0
" 17	1816	"	"	"	23.5	32.0	17.0
" 17	1816	"	"	"	22.1	31.0	16.0
" 18	1816	"	"	"	18.8	23.0	17.0
					25.8	36.0	16.0

CHART 34.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *Trypanosoma gambiense*, Tanganyika, Strain II., taken on 9 consecutive days from Rat 1816



This curve is very different from the other two. It may be noted that the blood of Rat 1816 was swarming with *T. lewisi*, but whether this had any effect on the curve it is impossible to say. Compare this curve with that of Chitukula, Chart 7.*

Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all.

Table CXLVII.—Measurements of the Breadth of *Trypanosoma gambiense*, Tanganyika, Strain II., measured across the widest part, including the undulating membrane.

Expt. No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
1816	Rat	500	2.66	4.75	1.50

Posterior-nuclear forms.—0 per cent.

* "Proceedings" of the Royal Society, B, vol. 86, 1913, p. 292, also Reports of the Sleeping Sickness Commission, No. XV., p. 39.

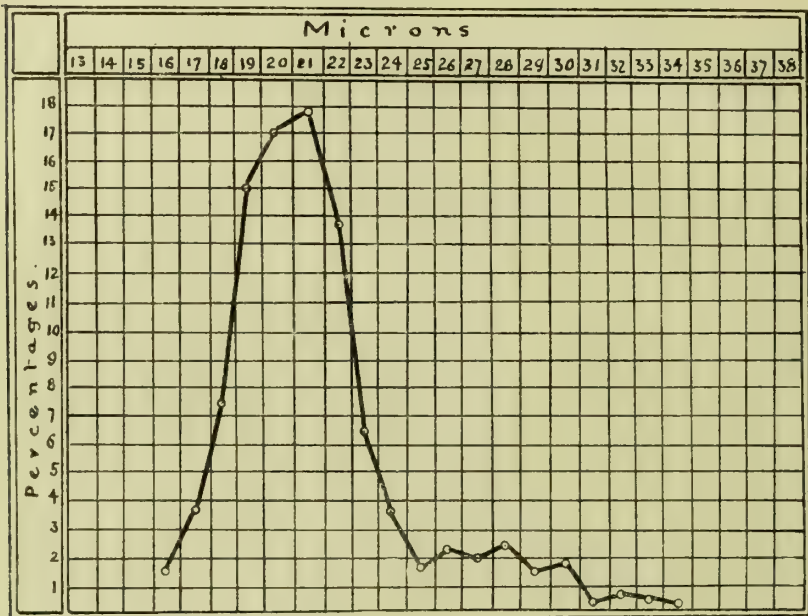
Morphology of Trypanosoma gambiense, Tanganyika, Strain III.

Length.—The following table gives the length of the trypanosome of Strain III., as found in the white rat—500 trypanosomes in all.

Table CXLVIII.—Measurements of the Length of *T. gambiense*, Tanganyika, Strain III.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Feb. 11	1820	Rat	Osmic acid	Giemsa	20·1	29·0	18·0
" 11	1820	"	"	"	21·0	29·0	17·0
" 11	1820	"	"	"	21·5	30·0	17·0
" 12	1820	"	"	"	20·9	30·0	17·0
" 12	1820	"	"	"	20·7	20·6	18·0
" 12	1820	"	"	"	21·1	30·0	18·0
" 13	1820	"	"	"	18·0	22·0	16·0
" 13	1820	"	"	"	18·5	21·0	16·0
" 13	1820	"	"	"	19·0	21·0	17·0
" 21	1820	"	"	"	22·9	29·0	17·0
" 21	1820	"	"	"	23·3	30·0	17·0
" 21	1820	"	"	"	24·4	32·0	18·0
" 22	1820	"	"	"	21·6	32·0	16·0
" 22	1820	"	"	"	21·4	28·0	17·0
" 22	1820	"	"	"	21·3	28·0	18·0
" 24	1820	"	"	"	23·0	32 0	18·0
" 24	1820	"	"	"	21·1	25·0	18·0
" 24	1820	"	"	"	22·2	26·0	20·0
" 25	1820	"	"	"	21·0	24·0	18·0
" 25	1820	"	"	"	21·6	31·0	17·0
" 25	1820	"	"	"	21·4	24·0	19·0
" 26	1820	"	"	"	22·0	33·0	18·0
" 26	1820	"	"	"	22·0	34 0	17·0
" 26	1820	"	"	"	21·6	29·0	18·0
" 27	1820	"	"	"	21·6	27·0	17·0
					21·3	34·0	16·0

CHART 35.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *Trypanosoma gambiense*, Tanganyika, Strain III., taken on 9 consecutive days from Rat 1820.



Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all.

Table CXLIX.—Measurements of the Breadth of *Trypanosoma gambiense*, Tanganyika, Strain III., measured across the widest part, including the undulating membrane.

Expt. No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
1820	Rat	500	2.92	4.50	1.25

Posterior-nuclear forms.—0 per cent.

Comparison of the Tanganyika Strains with One Another.

Table CL.—Measurements of the Length of the three Tanganyika Strains of *Trypanosoma gambiense*.

Date.	Expt. No.	Strain.	Animal.	No. of trypanosomes measured.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913	1866	I.	Rat	500	22·7	34·0	15·0
1913	1816	II.	"	500	25·8	36·0	16·0
1913	1820	III.	"	500	21·3	34·0	16·0
					23·2	36·0	15·0

Table CLI.—Measurements of the Breadth of the Trypanosome of the three Tanganyika Strains.

Date.	Expt. No.	Strain.	Animal.	No. of trypanosomes measured.	In microns.		
					Average breadth.	Maximum breadth.	Minimum breadth.
1913	1866	I.	Rat	500	2·86	4·50	1·25
1913	1816	II.	"	500	2·66	4·75	1·50
1913	1820	III.	"	500	2·92	4·50	1·25
					2·81	4·75	1·25

Comparison of the Tanganyika Strain of *Trypanosome gambiense*, with the Human, Wild Game, and Wild *Glossina morsitans* Strains of the *Trypanosome* causing Disease in Man in Nyasaland.

Table CLII.—Average Length of the Human, Wild Game, and Wild *Glossina morsitans* Strains of the *Trypanosome* causing Disease in Man in Nyasaland, and *Trypanosome gambiense*, Tanganyika.

Strain.	No. of trypanosomes measured.	Animal.	In microns.		
			Average length.	Maximum length.	Minimum length.
Human	5500	Rat	23·5	38·0	14·0
Wild Game	2500	"	22·6	35·0	15·0
Wild <i>G. morsitans</i> ...	2500	"	22·6	35·0	15·0
<i>T. gambiense</i> , Tanganyika	1500	"	23·2	36·0	15·0

Table CLIII.—Average Breadth of the Human, Wild Game, and Wild *Glossina morsitans* Strains of the Trypanosome causing Disease in Man in Nyasaland, and *Trypanosoma gambiense*, Tanganyika.

Strain.	No. of trypanosomes measured.	Animal.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
Human	1500	Rat	2·6	5·0½	1·25
Wild Game	1500	"	3·2	5·75	1·50
Wild <i>G. morsitans</i> ...	1500	"	2·9	5·25	1·25
<i>T. gambiense</i> , Tanganyika	1500	"	2·8	4·75	1·25

Conclusions.

1. *Trypanosoma gambiense*, Tanganyika, is very similar in morphology to *T. brucei vel rhodesiense*, the trypanosome causing disease in man in Nyasaland, but it would appear to be possible to distinguish between them by the blunt-ended posterior-nucleated forms which are so common in the blood of rats infected by the latter and absent in rats infected by *T. gambiense*.

2. It would appear to be impossible at present to distinguish between the two by microscopic examination of the parasites as they occur in the blood of man.

XXXVIII.—THE SUSCEPTIBILITY OF ANIMALS TO *TRY-PANOSOMA GAMBIENSE*, TANGANYIKA. STRAINS I., II., and III.

Table CLIV.

Strain I.

Date.	No. of expt.	Source of virus.	Period of incuba- tion, in days.	Dura- tion of disease, in days.*	Remarks.	
Goat.						
1913.						
Feb. 12	1867	From Monkey	1800	—	—	Never showed try- panosomes.
" 12	1868	" "	1800	—	—	" " "
Mar. 1	1867	From Rat	1819	—	—	" " "
" 1	1868	" "	1819	—	—	" " "
Jun. 11	2221	From Monkey	2113	15	—	Alive after 271 days.
" 11	2222	" "	2113	—	—	Never showed try- panosomes.
July 4	2263	" "	1800	—	—	" " "
" 4	2264	" "	1800	—	—	" " "
" 4	2265	" "	1800	—	—	" " "
Monkey.						
1912.						
Nov. 19	1800	From Man. Tan- ganika	?	264	Died of Strain I.	
1913.						
Feb. 12	1860	From Monkey	1800	—	—	Never showed try- panosomes.
" 12	1861	" "	1800	—	—	" " "
" 28	1860	From Rat	1819	16	—	Alive after 374 days.
Mar. 1	1972	" "	1819	19	—	Alive after 373 days.
April 26	2113	Lab.-bred Flies	...	?	171	Transmission Expt.
May 28	2192	From Monkey	2113	12	—	Alive after 285 days.
" 28	2193	" "	2113	12	226	Died of Strain I.
" 28	2194	" "	2113	12	217	" " "
" 28	2195	" "	2113	12	—	Alive after 285 days.
Nov. 27	2441	Lab.-bred Flies	...	?	46	Transmission Expt.
Average			...	13.8	184.8	
Dog.						
Feb. 12	1862	From Monkey	1800	—	—	Never showed try- panosomes.
" 12	1863	" "	1800	—	—	" " "
Mar. 1	1862	From Rat	1819	—	—	" " "
" 1	1863	" "	1819	19	180	Died of Strain I.
Jun. 11	2223	From Monkey	2113	15	124	" " "
" 11	2224	" "	2113	15	28	" " "
July 1	2249	" "	1972	23	53	" " "
Average			...	18.0	96.2	

* Duration includes the days of incubation; it dates from the day of inoculation.

Table CLIV. (continued).

Date.	No. of expt.	Source of virus.	Period of incuba- tion, in days.	Dura- tion of disease, in days.*	Remarks.
Guinea-Pig.					
1913.					
Feb. 12	1864	From Monkey	1800	—	Never showed try- panosomes.
Mar. 1	1864	From Rat	1819	—	" " "
July 4	2259	From Monkey	1800	—	" " "
" 4	2260	" "	1800	—	" " "
" 4	2261	" "	1800	—	" " "
" 4	2262	" "	1800	—	" " "
Rat.					
1913.					
Jan. 28	1818	From Monkey	1800	9	Died of Strain I.
" 28	1819	" "	1800	16	Killed 1st Mar. 1913.
Feb. 12	1865	" "	1800	—	Never showed try- panosomes.
" 12	1866	" "	1800	—	" " "
Mar. 1	1865	From Rat	1819	23	Died of Strain I.
" 1	1866	" "	1819	26	" "
May 17	2170	From Monkey	2113	12	" "
" 17	2170	" "	2113	23	" "
July 4	2256	" "	1800	17	" "
" 4	2257	" "	1800	—	Never showed try- panosomes.
" 4	2258	" "	1800	24	Died of Strain I.
Dec. 12	2452	" "	2441	6	" "
Average			...	17·3	114·7

Table CLV.
Strain II.

Date.	No. of expt.	Source of virus.	Period of incuba- tion, in days.	Dura- tion of disease, in days.*	Remarks.
Goat.					
1913.					
Feb. 12	1876	From Monkey	1801	—	Never showed try- panosomes.
" 12	1877	" "	1801	—	" "
Monkey.					
1912.					
Nov. 19	1801	From Man. Tan- ganyika	?	—	Inoculated at Uddjidi. Alive after 475 days.
1913.					
Feb. 12	1869	From Monkey	1801	—	Never showed try- panosomes.
" 12	1870	" "	1801	—	" "

* Duration includes the days of incubation; it dates from the day of inoculation.

Table CLV. (continued).

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Dog.					
1913.					
Feb. 12	1871	From Monkey 1801	—	—	Never showed trypanosomes.
„ 12	1872	„ „ 1801	—	—	„ „
Guinea-pig.					
Feb. 12	1873	From Monkey 1801	—	—	Never showed trypanosomes.
Rat.					
Jan. 28	1815	From Monkey 1801	—	—	Never showed trypanosomes.
„ 28	1816	„ „ 1801	9	85	Died of Strain II.
Feb. 12	1874	„ „ 1801	—	—	Never showed trypanosomes.
„ 12	1875	„ „ 1801	—	—	„ „

Table CLVI.

Strain III.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Goat.					
1913.					
Feb. 12	1885	From Monkey 1802	—	—	Never showed trypanosomes.
„ 12	1886	„ „ 1802	—	—	„ „
Mar. 1	1885	From Rat 1821	—	—	„ „
„ 1	1886	„ „ 1821	—	—	„ „
July 4	2273	From Monkey 1802	—	—	„ „
„ 4	2274	„ „ 1802	—	—	„ „
„ 4	2275	„ „ 1802	—	—	„ „

* Duration includes the days of incubation; it dates from the day of inoculation.

Table CLVI. (continued).

Date.	No. of expt.	Source of virus.	Period of incuba- tion, in days.	Dura- tion of disease, in days.*	Remarks.
Monkey.					
1912. Nov. 19	1802	From Man. Tan- ganyika	?	—	Inoculated at Udjidji. Alive after 475 days.
1913. Feb. 22	1878	From Monkey	1802	—	Never showed try- panosomes.
" 22	1879	" "	1802	—	" "
Mar. 1	1878	From Rat	1821	23	Died of Strain III.
" 1	1879	" "	1821	9	Alive after 373 days.
Dog.					
Feb. 12	1880	From Monkey	1802	—	Never showed try- panosomes.
" 12	1881	" "	1802	—	" "
Mar. 1	1880	From Rat	1821	—	" "
" 1	1881	" "	1821	—	" "
Guinea-pig.					
Feb. 12	1882	From Monkey	1802	—	Never showed try- panosomes.
Mar. 1	1882	From Rat	1821	26	Died of Strain III.
July 4	2266	From Monkey	1802	—	Never showed try- panosomes.
" 4	2267	" "	1802	—	" "
" 4	2268	" "	1802	—	" "
" 4	2169	" "	1802	—	" "
Rat.					
Jan. 28	1820	From Monkey	1802	13	Died of Strain III.
" 28	1821	" "	1802	16	Killed 1st Mar. 1913.
Feb. 12	1883	" "	1802	—	Never showed try- panosomes.
" 12	1884	" "	1802	—	" "
Mar. 1	1883	From Rat	1821	9	Alive after 373 days.
" 1	1884	" "	1821	26	" 373 "
July 4	2270	From Monkey	1802	17	" 249 "
" 4	2271	" "	1802	—	Never showed try- panosomes.
" 4	2272	" "	1802	—	" "

* Duration includes the days of incubation; it dates from the day of inoculation.

Table CLVII.—The Average Duration, in Days, of the Disease in Various Animals caused by *Trypanosoma gambiense*, Tanganyika.

	Monkey.	Dog.	Guinea-pig.	White rat.
Average duration, in days	159	96	264	137
No. of animals employed	6	4	1	10

Compare this with the following table.

Table CLVIII.—The Average Duration of Life, in Days, of Various Animals Infected with the Human Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Monkey.	Dog.	Guinea-pig.	White rat.
Average duration, in days	26	34	67	30
No. of animals employed	20	25	15	21

Conclusions.

1. It is at first a matter of some difficulty to infect goats and other experimental animals with *Trypanosoma gambiense*. But it would appear that after the trypanosome has become accustomed to any particular species this difficulty disappears to a great extent.

2. The disease in animals caused by *Trypanosoma gambiense* is much more chronic than that caused by *Trypanosoma brucei vel rhodesiense*.

XXXIX.—THE DEVELOPMENT IN *GLOSSINA MORSITANS* OF *TRYPANOSOMA GAMBIENSE*, TANGANYIKA.

This trypanosome belongs to the *T. brucei* group, in which the development takes place in the alimentary canal, whence the trypanosomes find their way into the salivary glands. Here they take on the "blood form" and become infective.

Twelve experiments were carried out with laboratory-bred flies. Two were positive and ten negative.

Table CLIX.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1913.						
Jan. 24	1804	33	--	1	-	84° F. (29° C.)
Jan. 30	1825	38	—	3	-	"
Feb. 26	1965	45	--	3	-	"
Mar. 5	1979	50	+	2	64	"
April 23	2107	60	--	0	-	"
May 12	2132	40	---	0	-	"
May 19	2173	30	--	1	-	"
June 6	2208	43	--	2	-	"
Sept. 15	2411	46	---	0	-	"
Sept. 29	2416	40	--	0	-	"
Oct. 6	2420	55	+	0	50	"
Nov. 3	2428	25	--	0	-	"

Five hundred and five flies were used. Twelve infected flies were found—2·3 per cent. It is probable, however, that some infected flies escaped detection, as few or none of the flies used in the last four experiments were examined. Dr. Taute, on Lake Tanganyika, in 670 flies found at least 10 infective—1·5 per cent.—and in 282 flies examined microscopically, developmental forms were found 22 times—7·8 per cent.

Details of the Two Positive Experiments.

The following table gives the details in the carrying out of the two positive experiments:—

Table CLX.

Expt.	Day of expt.	Procedure.	Remarks.
1979	1—10	Flies fed on infected monkey	Trypanosomes first appeared in blood of monkey 2113 sixty-four days after first infected feed. All flies dissected; two found infected
	11	Starved	
	12—50	Fed on clean dog 1992.	
	51	Starved.	
	52—66	Fed on clean monkey 2113.	
2420	1—10	Flies fed on infected monkey	Monkey 2421 became infected on the 2nd December, 1913, 50 days after the infected feed. No infected fly was found.
	11	Starved	
	12—54	Fed on clean monkey 2421	

It would appear from these two positive experiments that a period of 50 or 60 days may elapse before the cycle of development of *T. gambiense* in *G. morsitans* is complete and the "fly" becomes infective.

Conclusions.

It would appear that *Trypanosoma gambiense*, under the climatic conditions obtaining at Kasu camp, experienced more difficulty in developing in *Glossina morsitans* than did *Trypanosoma brucei* vel *rhodesiense*. Out of 12 experiments, only two were positive, and the flies took a long time before they became effective.

F.—TRYPANOSOMES AND OTHER PARASITES OF ANIMALS IN NYASALAND.

XL.—*PLASMODIUM CEPHALOPHI*, SP. NOV.*

It would appear from a perusal of the available literature that malaria of antelopes has not hitherto been described; it is therefore proposed to place on record the discovery of a plasmodium found in the blood of two of these animals in Nyasaland.

This parasite was first seen in the blood of a young duiker (*Cephalophus grimmii*), and was subsequently discovered in another young animal of the same species. Both these small antelope were at the time in captivity, and it was therefore possible to examine slides from day to day, and by this means a large number of parasites at various stages of development were observed, and some of these are figured in Plates 12 and 13.

The acute attack in one antelope, however, only lasted four days, and the parasites soon disappeared entirely from the peripheral blood, whereas, in the other, only a few parasites were seen, and these have persisted in the blood for some months.

The parasites resemble somewhat *Plasmodium malariae* of man, in that the gametocytes are circular and the schizonts have from eight to twelve merozoites; also amoeboid movement is sluggish. They differ, however, in the marked enlargement and paleness of the red cell and in the arrangement and appearance of the pigment, which is not scattered throughout the parasite, but is collected in a single mass, and is pale yellow in colour (Plate 13). Schüffner's dots have never been seen, but in some instances (Plate 12, figs. 5, 6, and 7) pseudopodia were seen with a scar in the red cell recalling the formation of Maurer's dots in *Laverania malariae* of man. The forms of trophozoite shown in Plate 12, figs. 6 to 12, are, so far as our experience goes, unique, first in the dense blue-staining (Giemsa) of the protoplasm, and, secondly, the great enlargement and marked paleness of the red cell with absence of Schüffner's dots, and the intense

* Reprinted from the "Proceedings" of the Royal Society, B, vol. 87, 1913, p. 45.

concentration of the portion of red cell in the food vacuole. This concentration, as will be seen in the figures, proceeds until nothing is left but a block of gamboge-yellow pigment (Plate 12, figs. 14 to 16). The distended red cells are so delicate that they are frequently ruptured in making the film, as is shown in Plate 13, figs. 7 and 8. The great size of the parasite is also remarkable, some of the free merozoites measuring 4 microns by 3.5 microns, the exact size of a normal red cell of the antelope. A full grown schizont, before cleavage of the protoplasm, measures 10 microns by 10 microns (Plate 12, fig. 19).

The presence in the gametocytes of numerous deeply staining chromatin granules, in addition to the faintly stained nucleus, is also remarkable. The arrangement of these granules in some of the merozoites suggests a relationship with the *Hamoflagellata* (Plate 12, figs. 3 and 23); and in this connection it may be mentioned that one duiker was also naturally infected with a trypanosome of non-pathogenic type, which it is proposed to describe in a future paper.*

In films taken some days and even months after the acute attack, the forms shown in Plate 13, figs. 12 to 16, are common, and but for the presence of pigment might be mistaken for faintly stained leucocytes. As these are the most persistent forms (macrogametocytes), attention is directed to them, as they are the forms likely to be met with in examination of blood films of antelope taken in the field.

One of the small duikers appeared out of condition for a few days, while the parasites were numerous in the blood: coat staring, nose hot and dry; but it soon regained its health, and has remained healthy since. The other duiker was apparently not at all affected, although a few parasites could be found in its blood for some six months.

The duiker which was chronically infected was captured in the low country near the Lake shore, and was about three months old when brought to Kasu Hill, where a few weeks later a few parasites were found in its blood. It is therefore probable that it was infected before capture. Whereas the other antelope was caught on the plateau and had been in captivity and not in the vicinity of other wild game for at least six months before it was brought to Kasu. As it developed an acute attack a few weeks after it had been placed in the same enclosure with the infected duiker, it would appear that in its case the infection was contracted locally.

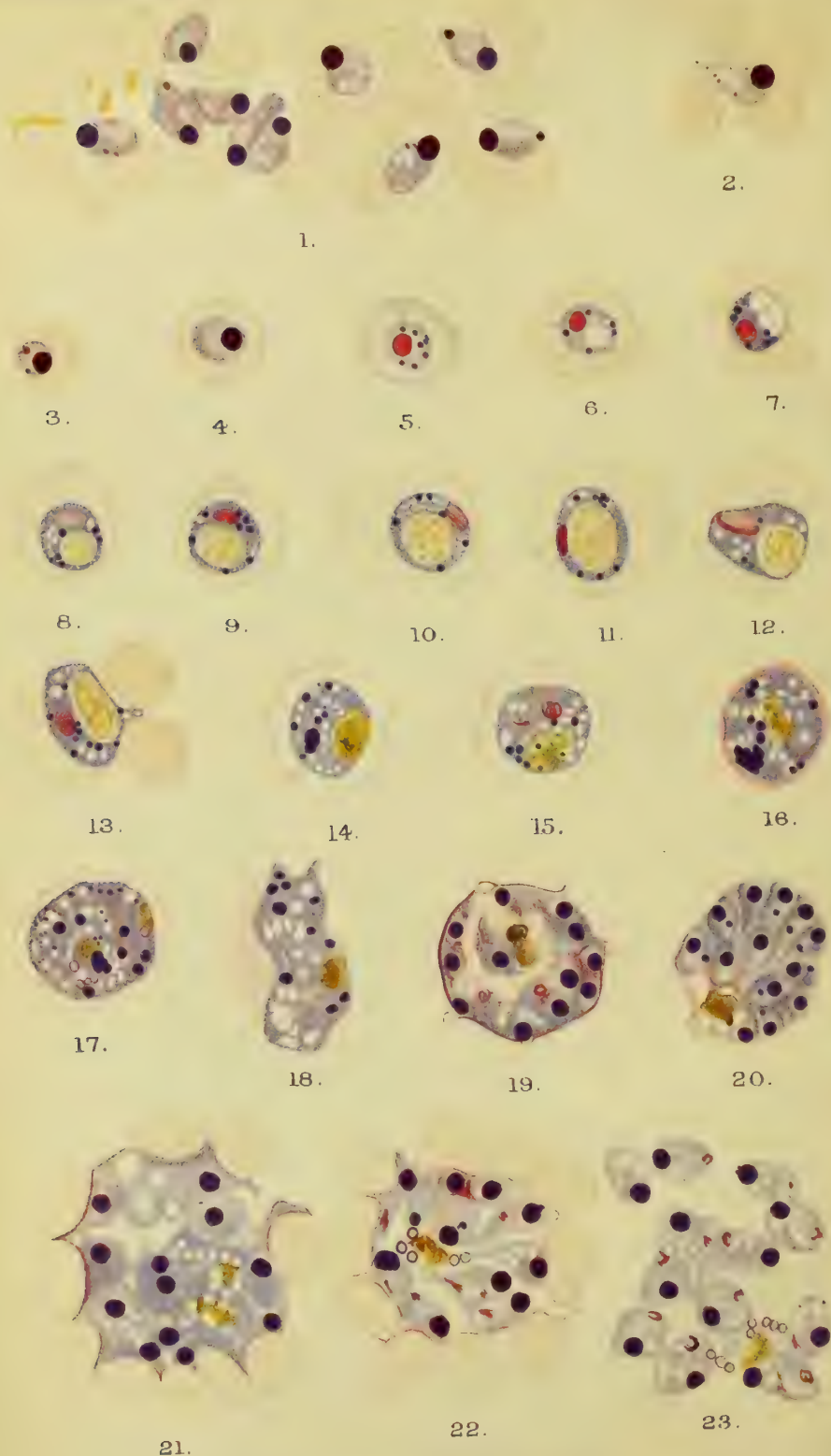
The two antelope were first found to be infected in the height of the dry season (October), and although a systematic search was made for adult or larval mosquitoes, none could be trapped or found in or near the enclosure.

In the same compound there were also another young duiker, a young reed buck, and a young hartebeeste, and although the blood of these animals was examined frequently, no malarial parasites could be found.

If this parasite should prove to be a new one, the name of *Plasmodium cephalophi* is proposed for it.

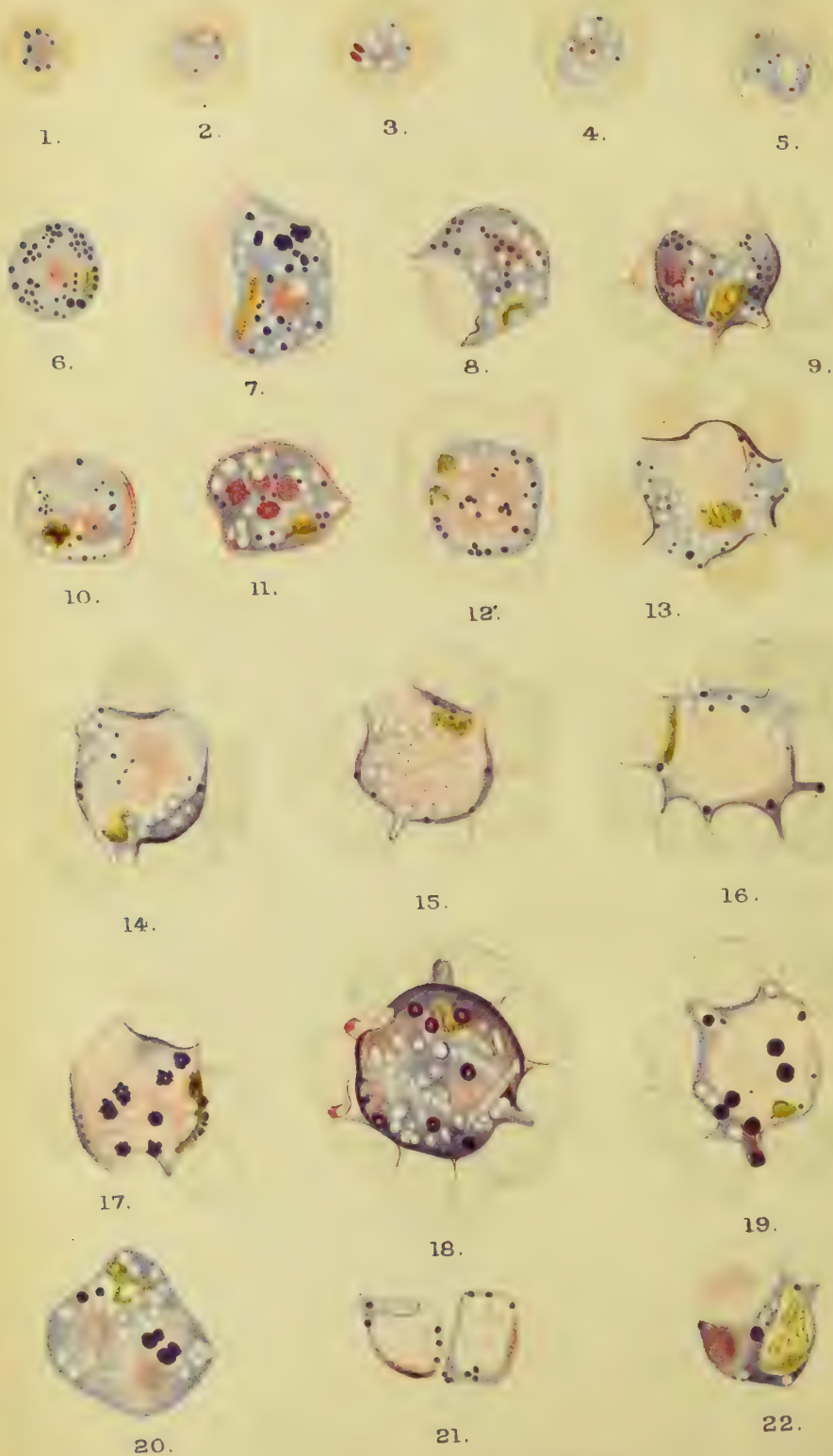
* See p. 205 of this Report.





Plasmodium cephalophi. sp. nov.





Plasmodium cephalophi sp. nov.

M.E. Bruce del.

Huth, London

DESCRIPTION OF PLATES.

PLATE 12.

- Fig. 1.—Schizont, ruptured in making the film. The merozoites are drawn along in the direction of the spreading of the blood. The residual mass and free pigment are also shown.
- Figs. 2 and 3.—Merozoites entering red cell. Note the granules in fig. 2 scattered throughout the protoplasm; in fig. 3 there are two granules, one large and one small.
- Figs. 4-15.—Trophozoites, showing marked enlargement and paleness of red cells and concentration of portion of red cell in vacuole.
- Figs. 16-18.—Young forms of "rosette" formation.
- Fig. 19.—Fully developed schizont before cleavage of protoplasm.
- Figs. 20-22.—Schizonts which show commencing cleavage of protoplasm, with a single mass of gamboge-coloured pigment.
- Fig. 23.—Schizont in which the merozoites are completely separated. Some are oval and some circular in shape. All show a V-shaped group of granules at the opposite pole from the nucleus.

Stained Giemsa, $\times 2000$.

PLATE 13.

- Figs. 1-5.—Young parasites, with faintly staining protoplasm and without vacuole. These were found in the same films as the forms shown in Plate 12, figs. 6-14.
- Figs. 6-11.—Gametocytes, with faintly staining nucleus and granular protoplasm.
- Figs. 12-16.—Forms found in the blood some weeks after acute attack (macrogametocytes).
- Figs. 17-22.—Some anomalous forms.

Stained Giemsa, $\times 2000$.

XLI.—*TRYPANOSOMA CEPHALOPHI*, S.P. NOV.

This trypanosome was found in the blood of two duikers (*Cephalophus grimmi*) in October, 1912. As it does not seem to have been described, the name *Trypanosoma cephalophi* is suggested.

It was very scanty, only four specimens being found after a long search in the blood of both antelope. One was 40 microns long and 6 broad; another, 50 long and 5 broad; the third, 40 by 5; and the last, 41 by 4. The measurement of the breadth does not include the undulating membrane.

An attempt was made to cultivate this trypanosome from the blood of the duiker. Five tubes were inoculated. Only one of these showed any growth. After eleven days numerous actively motile herpetomonad flagellates were present. At the end of a week these

had died off. Sub-cultures were made from the positive tube, but these showed a very scanty or no growth and also soon died off.

Plate 14, figs. 1, 2, and 3, represent this trypanosome.

XLII.—*TRYPANOSOMA PETRODROMI*, SP. NOV.

This trypanosome was found in the blood of a jumping shrew (*Petrodromus venustus*). It measures about 36 microns, on an average, and about 5 in breadth. The protoplasm stains evenly, and no myonemes were observed. The nucleus stains well, is oblong with rounded corners in shape, the long axis lying transversely and somewhat obliquely to the long axis of the trypanosome; as a rule the nucleus lies towards the anterior or flagellar end. The micronucleus is large and round, about 1 micron in diameter, and placed about 5 microns from the posterior extremity, which is elongated and tapers to a blunt or rounded end. The undulating membrane is narrow, and the flagellum is terminated by a nodule very similar in appearance to that described by Mesnil and Brimont* in *Trypanosoma legeri* from an antbear in South America.

Plate 14, figs. 4 to 9, represent this trypanosome.

XLIII.—*FILARIA* OF JUMPING SHREW.

These filariæ were common in the blood of the jumping shrew. The adult worms were found in one case lying in tight coils between the muscles on either side of the neck, two on each side. They were about the thickness of very fine sewing cotton, nearly two inches in length, and opaque-white in colour.

The embryos are figured in Plate 15, figs. 1 and 2.

XLIV.—*PLASMODIUM* OF JUMPING SHREW.

This was common in the blood of the jumping shrew. The only forms seen were those figured in Plate 16, figs. 16-19, and evidently show a marked resemblance to the gametes seen in the blood of monkeys suffering from malaria.

XLV.—*PIROPLASM* OF EDIBLE RAT.

This piroplasm was observed in the blood of the edible rat. It somewhat resembles the piroplasm found in the blood of a white mouse in Uganda.†

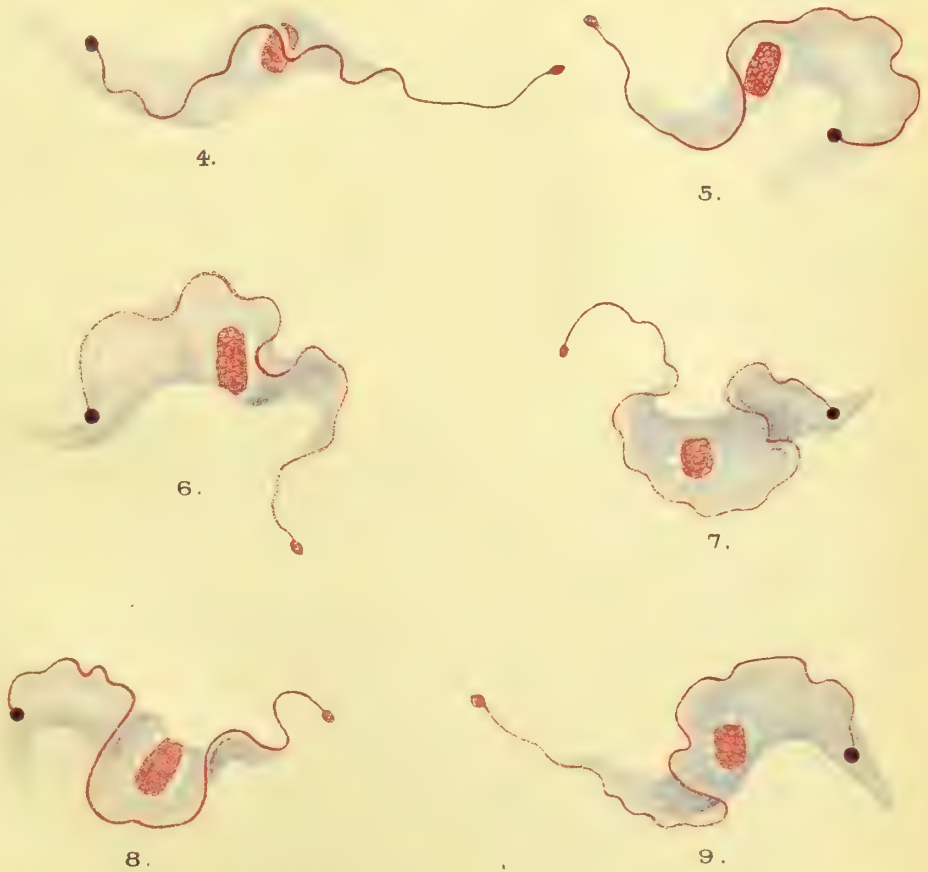
Plate 16, figs. 20-29.

* *Soc. de Biologie*, 16th July, 1910.

† Reports of the Sleeping Sickness Commission, No. XI., p. 189.



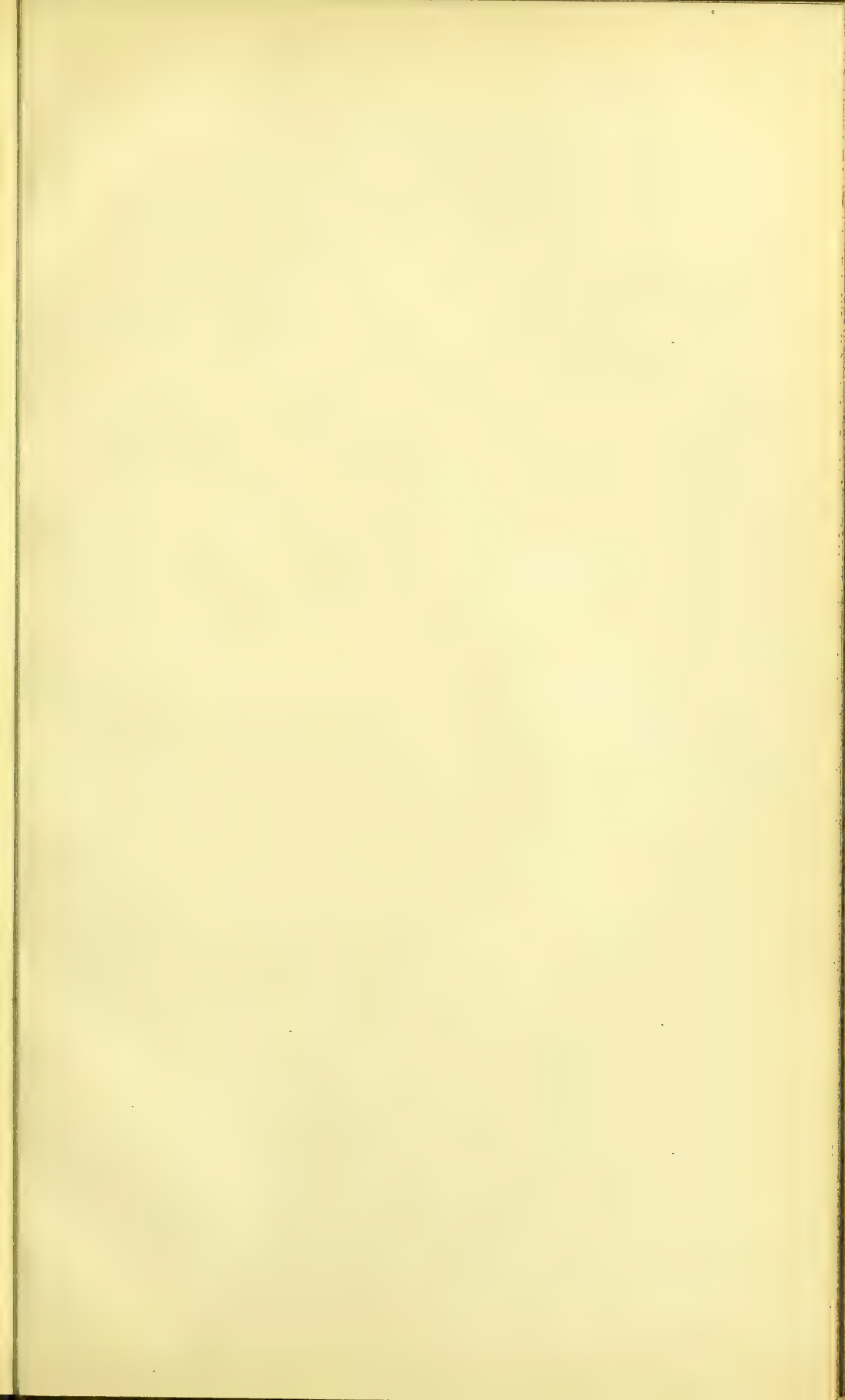
Trypanosoma cephalophi.



Trypanosoma petrodromi.

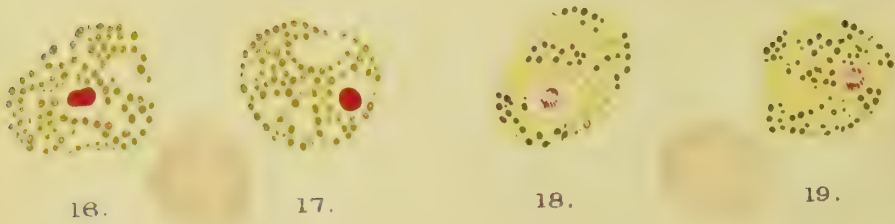


Filaria from various Animals. Nyasaland.





Plasmodium of Antelope.



Plasmodium of Jumping Shrew.



Piroplasm of Edible Rat.

X 2000.

Huth, London.

XLVI.—FILARIA OF ANTELOPE.

These filariæ were found in the blood of koodoo, waterbuck, hartebeeste, and Sharp's steinbuck. The adult worm was not found. Plate 15, fig. 3.

Another species of filaria (Plate 15, fig. 5) was found in the blood of the hartebeeste and oribi.

XLVII.—FILARIA OF WARTHOG.

The blood of the warthog also commonly showed filaria (Plate 15, figs. 6 and 7). The adult worm was not found.

XLVIII.—PLASMODIUM OF ANTELOPE.

What appears to be a species of plasmodium differing from *Plasmodium cephalophi* was also found in two duikers. The various forms seen are figured on Plate 16, figs. 1 to 15.

XLIX.—FILARIA OF DOGS AND MONKEYS.

Filaria embryos were often observed in dogs and monkeys. Those found in the dog belonged to the species *Filaria immitis*, and those in the monkey were similar to those seen in monkeys in Uganda.

Plate 15, fig. 4.

APPENDIX I.

Summary of temperature observations recorded at Kasu, Central
Angoniland, Nyasaland (lat. 13° 40' S., long. 34° 12' E.).
3,500 feet above sea level.

Month.	Temperature.		Range.	Mean Max.	Mean Min.	Mean Tem- perature.
	Max.	Min.				
1912.	°	°	°	°	°	°
January* ...	77	65	12	74·3	67·3	70·8
February ...	76	60	16	73·2	66·6	69·9
March ...	77	61	16	72·7	66·9	69·8
April ...	76	64	12	73·8	68·2	71·0
May ...	75	62	13	72·2	66·8	69·5
June ...	73	57	16	68·2	61·5	64·8
July ...	68	56	12	64·6	59·5	62·0
August ...	72	57	15	68·6	62·6	65·6
September ...	78	63	15	73·5	67·9	70·7
October ...	81	63	18	76·7	69·2	72·9
November ...	84	70	14	79·8	74·4	77·1
December ...	78	68	10	74·2	70·2	72·1
1913.						
January ...	76	66	10	72·5	69·0	70·7
February ...	78	68	10	74·0	70·2	72·1
March ...	75	66	9	71·7	68·8	70·2
April ...	74	65	9	71·9	68·3	70·1
May ...	73	62	11	70·6	65·4	68·0
June ...	69	57	12	65·3	60·8	63·0
July ...	71	58	13	66·1	61·7	63·9
August ...	74	61	13	69·5	64·0	66·7
September ...	76	63	13	73·0	67·8	70·4
October ...	81	67	14	77·1	71·5	74·3
November ...	82	70	12	78·9	74·2	76·5
December ...	82	68	14	77·6	73·1	75·3
1914.						
January ...	77	68	9	74·6	70·7	72·6
February ...	76	68	8	73·5	70·4	71·9
March ...	75	68	7	73·2	69·7	71·4

* Readings commenced January 20th, 1912, and ceased March 17th, 1914. Carried out 9 a.m. and 2 p.m. daily in the office. This was a brick building, with thickly thatched roof, and corresponded fairly well with the temperature of the laboratory.

Observer: JAS. WILSON.

APPENDIX II.

DURATION OF PUPATION OF *GLOSSINA MORSITANS*.

A number of tsetse flies were kept in captivity, in order to obtain pupæ from which to breed clean laboratory-bred flies. A good deal of difficulty was met with. It was impossible to spare a white man from Kasu to superintend the native fly-catchers in the "low-country." Without such supervision it is hard to get the native to work. Again, at Kasu the conditions of temperature and humidity did not seem to agree with the flies, so that it was necessary to set up a breeding place in the "fly-area" among their natural surroundings. Here, of course, there was less control over the natives than ever. The pupæ were brought up to Kasu and hatched there.

In the following Table is given the number of pupæ deposited each month from September, 1912, until June, 1913; the proportion of male pupæ to female pupæ (this works out at eight males to seven females); the duration of pupation (this varies with the temperature); the percentage of the pupæ which hatched out; and, lastly, the mean temperature of each month.

Date.	Pupæ deposited.	Pupæ hatched.		Total hatched.	Duration of pupation in days.	Per-centage of pupæ hatched.	Mean tem-perature.
		♂	♀				
1912							
September...	65	28	28	56	35.1	86.2	70.7
October ...	75	33	31	64	30.1	85.3	72.9
November...	292	102	101	203	31.1	69.5	77.1
December ...	465	187	148	335	34.3	72.0	72.1
1913							
January ...	260	116	89	205	35.3	78.8	70.7
February ...	253	110	82	192	36.0	75.9	72.1
March ...	192	92	49	141	39.0	73.4	70.2
April ...	230	76	83	159	44.5	69.1	70.1
May ...	136	39	46	85	63.1	62.5	68.0
June ...	134	24	40	64	68.7	47.8	63.0

APPENDIX III.

REPORT ON CERTAIN INVESTIGATIONS IN THE
ELEPHANT MARSH, RUO DISTRICT.

By Dr. J. E. S. Old, Nyasaland Medical Staff.

On my return from leave in April, 1912, I found a letter from the Principal Medical Officer, containing a request from the Director of the Scientific Commission of the Royal Society, through His Excellency the Governor, that I would put myself in communication with him. This I did, and I was asked that if I could find the time and a sufficient number of *Glossina brevipalpis* in my district, to try whether (a) laboratory-bred flies would convey *Trypanosoma brucei* vel *rhodesiense* from an infected animal to a healthy one; (b) would wild *G. brevipalpis* convey any trypanosome disease to healthy monkeys and goats if fed on them? and (c) would an ox exposed to the bites of this tsetse fly show trypanosomes in its blood? If (c) proved successful, I was to inoculate a couple of dogs from the ox and send them to Kasu.

I promised to endeavour to carry out experiments (b) and (c), but as my experimental camp would be at least 45 miles by road from my headquarters, and I could not leave any responsible person in charge when absent from it, I did not feel justified in risking experiment (a).

I left Chiromo on the evening of September 11th, and the next evening found a suitable camping place near Mlolo's village, Muona, in forest, one hundred yards from the Tangasi stream, and apparently a most suitable locality for tsetse flies. There I built a kraal some fifty yards from my tent.

Immediately I started hunting for *Glossina brevipalpis* at the Tangasi stream and its vicinity, from where it emerged from the hills to where it disappeared in the plain as a marsh at Gwada. Special attention was paid to the sunrise and sunset hours for this work in view of the known habits of this tsetse fly. Not a single specimen was heard or seen by me. A specimen shown to the headman and other natives proved their ignorance of the existence of such a fly.

Tsetse records.—The only recorded captures of tsetse at Muona, so far as I was aware, were one by Mr. S. A. Neave, when he examined the locality about 1910, I think at the stream on the Chiromo-Masengeri road, and one by Mr. Ranald MacDonald in August, 1911, when crossing the dry bed of a stream on the Chikonje-Muona-Masengeri road. This latter one I sent to the Entomological Research Committee. Both these spots were within a mile of my camp.

Character of Stream and Country.—Practically the whole length of this stream, to the extent stated above, is thickly fringed with banana trees, has some very large shade trees here and there, and has shady forest, with generally very long grass, to within 200 yards of it, on an average, until it becomes a marsh. The stream remained clear running water, with sandy or muddy-bottom pools.

throughout this exceptionally dry year, as far as the Chiromo road, beyond which, in October, the marsh entirely dried up, and wells were dug by the villagers in the bed of the stream, which was planted with maize and pumpkins.

Game.—Game of some kind visited most parts of the stream at night. Bushbuck, waterbuck, and hyænas were about my camp practically every night. The square mile where the stream diffused itself on the plain was occupied about midday daily by about 200 head of game in September; these were mostly reedbuck and waterbuck, with several zebra, and a few hartebeeste and warthog. A small herd of buffalo visited the same spot to feed on the green grass at night. As the water dried up, in October, the waterbuck and zebra practically disappeared, some at any rate having gone to the hills, as did also the herd of eland which had been coming to the stream at night.

Masengeri.—Anticipating now that I should not succeed in finding tsetse during this trip, I asked Mr. Milthorp, Resident, to purchase two goats for me in a non-tsetse locality—the Kampala, opposite Chiromo. On September 20th I proceeded to Masengeri. A female goat was sent to my Muona kraal, where it was carried off the second night by a leopard, and a male goat to Masengeri, where I used it as a tsetse bait.

Tsetse Fly Records.—So far as I am aware, the following are the only “fly” records at this place: (a) caught by Major F. B. Pearce, C.M.G., several years ago, and (b) one caught by myself in August, 1910, on a man’s chest at midday. These were caught on the high road, near the Rest House.

Game.—Game is not nearly so plentiful here as it was a few years ago, owing to so many sportsmen from the highlands, etc., visiting it, but a herd of 40 buffaloes and 30 elephants drank close to my tent the first night near the Rest House. Reedbuck are still pretty plentiful, and there was a herd of eland, but waterbuck, hartebeeste, zebra, and warthog were scarce.

Formation of Marsh.—The stream here is called Milolo. The stream at Tumbulendi is called Pala, and it diffuses on the plain at Mitondo. Between these two good streams is a less important one called Chisimbi. All these streams, with the Tangasi, produce the Elephant Marsh in the rains, the whole mass of water flowing towards, and entering, the Shiré river near Chirimo. The Milolo is usually a fine stream of cool water in normal years, but at the time of my visit it had dried up below the road, and there remained only wells. There was also a remarkable difference in the profuseness of reed-growth, much of this ground being in tillage. The thin open forest reaches practically to the banks of this stream, which soon diffuses itself over the open plain. The stream was examined at sunrise and sunset from where it emerges from the hills to the open plain, but no tsetse were seen, although a goat was used as a bait. My men were always constantly being reminded to watch each other’s backs for tsetse.

Blood Inoculation.—I decided to inject the blood of game into goats and dogs, to endeavour to isolate trypanosomes by this method, and I started with an eland on September 26th.

Blood Smears.—Thick and thin smears had been taken from the first of all game shot. The thin one was stained with Giemsa and examined by myself, and a thick one of each was sent to the Commission at Kasu.

On September 27th I returned to Muona to continue blood work.

From now *Tabanidæ* began to make their appearance, but with marked intervals, depending on weather conditions. More males than females were caught.

On October 17th there was a heavy thunderstorm, which was exceptionally early.

Tsetse Fly Records.—On November 6th, when bathing in my tent between 5 and 6 a.m., I caught a *Glossina brevipalpis* biting my wet thigh. This I consider the first of a new generation.

On November 13th I was ordered by His Excellency the Governor, by telegram, to return to Port Herald, and left the next day, thus terminating my work too abruptly. I was sorry for one reason, namely, that I had found a habitat of male *Tabanidæ*, which were beginning to appear in numbers.

I am indebted to Mr. E. T. Milthorp, Resident, for such assistance as the purchase of goats and loan of a native policeman.

Results.—I found no trypanosome of any kind in any animal, but in the thick smears I sent from game the Commission found the non-pathogenic species *Trypanosoma ingens* in two old animals, namely, a cow eland which I shot at Masengeri, and a ram bushbuck at Muona.

The blood of 28 head of game from the Elephant Marsh was examined microscopically, and by the inoculation of dogs and goats, but in no instance was a single pathogenic trypanosome seen by the Commission or myself.

Although this work is not exhaustive, it seems to show that trypanosome disease, if present in the Elephant Marsh, is not a very serious matter, and that *Glossina brevipalpis* is not nearly such a pest as *Glossina morsitans*. Also that the public outcry against it being preserved as a game reserve was unjustified.

In the exceptionally dry season of 1912 there were probably no tsetse flies between Chiromo and Masengeri in the months of September and October, that is after the grass had been largely burned, and, therefore, for all practical purposes, it should be safe to take cattle to or from Tumbulendi (Cholo hills) during these months.

Probably for several months in the year *Glossina brevipalpis* occurs at Muona and Masengeri, and also, no doubt, in suitable forest country between these points (ten or twelve miles) and in the direction of the Ruo (eight to ten miles).

Note by Director.—Besides the *Trypanosoma ingens* found in the blood of an eland and bushbuck, the Commission found *Trypanosoma pecorum* in a blood smear of a goat sent to Kasu by Dr. Old. This goat had been injected with blood from an eland, a reedbuck, and two bushbuck. It would appear from Dr. Old's investigations that *Glossina brevipalpis* was very scarce at the time of his visit, and that the wild animals were but slightly infected.

APPENDIX IV.

NOTES ON THE HABITS OF *G. MORSITANS*.

By Major A. E. Hamerton, D.S.O., R.A.M.C.

A message was received by the Commissioner in April, 1912, from Chief Matumba, of Matumba's village, requesting that someone would come and destroy a herd of elephants which were ravaging his maize crops. The writer was immediately despatched to Matumba's village, which lies about a mile off the Kasu-Domira Bay road, 19 miles from Kasu. The village is situated in the fly-area, sheltered by a clump of great forest trees, surrounded by a narrow circle of maize gardens, beyond which many square miles of almost impenetrable bush and grass jungle extends in all directions.

A herd of about 20 elephants, led by the proverbial savage old bull with enormous tusks, was said to visit the gardens every night and devastate the crops. On inspecting the gardens the tracks of four or five elephants were seen. About half an acre of corn had been trampled down, and the green crops of sweet potatoes planted between the maize had been eaten. The natives said that the elephants would return about nine o'clock that night, and would leave the gardens before daybreak. They did return about 10 p.m., but as there was no moon, shooting was out of the question, so they were frightened away by the beating of drums. They returned about 3 a.m., but left before dawn.

On following their tracks through the long grass, they were heard trumpeting about half a mile away. The native hunters said that at mid-day the elephants would stop to rest for an hour or two under a certain clump of big trees in a swampy place where the grass was very high, and that if we followed quickly we should overtake them. Their tracks were like narrow lanes meandering between high walls of bush and grass. Tsetse flies, which were very numerous all along the track, attacked us fiercely from daylight till noon. When leaving the tracks of the elephants we did not notice the attentions of these pests, and rarely saw one until coming again into the fresh tracks of elephants late in the afternoon, when the fly bothered us again until sunset. It was evident that swarms of tsetse flies frequented the fresh tracks of elephants, and that half a mile from their tracks fly were not noticeable. A few flies were caught, but none contained blood. It is probable that the flies conceal themselves immediately after feeding. About mid-day we caught up the elephants—an old female and calf and three or four half-grown beasts. They had been resting under a clump of trees in a tangled thicket of swamp grass and "wait-a-bit" thorns, which the elephants had tunnelled in all directions, as rats tunnel hay-fields in England. We could hear the brutes trumpeting and snorting as they passed uneasily along the tracks on either side of

us, for they had apparently got our wind and were moving on at the rate of about six miles an hour. Our efforts to cut them off amidst the intricate network of paths winding through the dense jungle were unavailing. The writer failed to catch even a momentary glimpse of one which apparently passed him within a score of yards. The natives could see them all from the tree tops, but not near enough to shoot. The elephants quickly passed out of the thicket into more open country, where they divided up into twos and threes, and went by separate tracks to the lake, whence, after watering, it was said, they would return by circuitous routes to the maize gardens.

A noticeable feature in the habits of *G. morsitans* is their fondness for following and settling upon rapidly-moving animals. A motor cyclist travelling through the fly-country at the rate of 20 or more miles an hour collects hundreds of tsetse flies on his back. In this way tsetse flies may be conveyed many miles and distributed over the country.

APPENDIX V.

NOTES ON THE HABITS OF *GLOSSINA MORSITANS*.

By Staff-Sergeant A. Gibbons, R.A.M.C.

September 30th, 1912.—I started from Chunzi* to Lingadzi with 20 fly-boys, each with nets and boxes. Only one fly caught before Mtalimanja's and Lingadzi. Very few flies were seen, although parties of boys were sent into the bush on either side of the road. Flies became fairly numerous on the made-up road across the marsh (Liwadzi river) before reaching the Lingadzi estate. It was found that nearly all the flies caught resting on the paths were males, whereas if I rode my cycle backwards and forwards, quite 50 per cent. of flies caught on my back were females. Hot sunny day with slight breeze. Result, 20 females, 56 males.

October 1st.—Started from Chunzi towards Matumba's, following the path through the bush. Flies were very scarce. Dull, cool, and very windy day. Result, 5 females, 32 males.

October 2nd.—Left Kasu for Matumba's; arrived 11 a.m. After passing through Matumba's village I met two of the fly-boys in the bush. They had caught, since early morning, about 30 male flies, but only one female. I took these boys with me to the Domira-Bay road, and while I rode my cycle they ran alongside and caught flies from my back. Of the first three flies caught two were females. This big percentage of females continued while they caught flies from off me when cycling. Hot sunny day.

October 3rd.—Sent the fly-boys out along the Domira-Bay road with instructions to catch flies in the bush on the left-hand side of the road. I followed and investigated the bush on the right-hand side of the road. Hot sunny day. Result, 5 females, 129 males.

October 4th.—Went along the road with the fly-boys about three miles beyond Ntongo's village, and then turned into the bush on the right. Very few flies seen until after four miles' walk, when the bed of a dried-up stream was reached. Flies were very numerous here, but the percentage of female flies was small. Hot sunny day. Result, 16 females, 158 males.

October 5th.—Took all the boys on the Domira-Bay road and separated them into two groups. One group to catch flies from the road and from one another's backs, the other group accompanied the cycle. The result of the morning's catch by the 14 boys separated from me was 4 females, 62 males; six boys with me catching flies from my back whilst cycling, 19 females, 35 males.

* The positions of villages and localities mentioned in these notes are shown on the Map at p. 1 of this Report.

APPENDIX VI.

REPORTS ON THE TSETSE-FLY AREAS OF THE NYASALAND PROTECTORATE IN THE YEAR 1913, AND ON THEIR INCREASE OR DECREASE SINCE THE YEAR 1909, TOGETHER WITH OBSERVATIONS ON THE INCREASE OR DECREASE IN BIG GAME DURING THE SAME PERIOD.

The following summary has been compiled by the Secretariat, Zomba, from Reports submitted by Residents, Nyasaland, and was forwarded to Kasu, with the original reports and maps, under cover of Acting Government Secretary's letter, No. 2354, dated November 11th, 1913.

I.—In accordance with the request of the Director of the Scientific Commission, the Residents in charge of the various Districts and Sub-Districts in the Protectorate were instructed to furnish a report dealing with the following matters:—

- (a) The extent of the existing tsetse-fly areas, distinguishing between belts of *Glossina morsitans* and other species of tsetse fly.
- (b) The increase or decrease, if any, in the tsetse-fly areas since the date of the report furnished in the year 1909.
- (c) The increase or decrease, if any, in big game since the date of the report furnished in the year 1909.

The instructions required each report to be accompanied by a sketch map illustrating (a) and (b) above.

II.—The following is a summary of the reports submitted by the Residents. Reference to the accompanying Map 3 should be made to ascertain the position of districts and localities mentioned below.

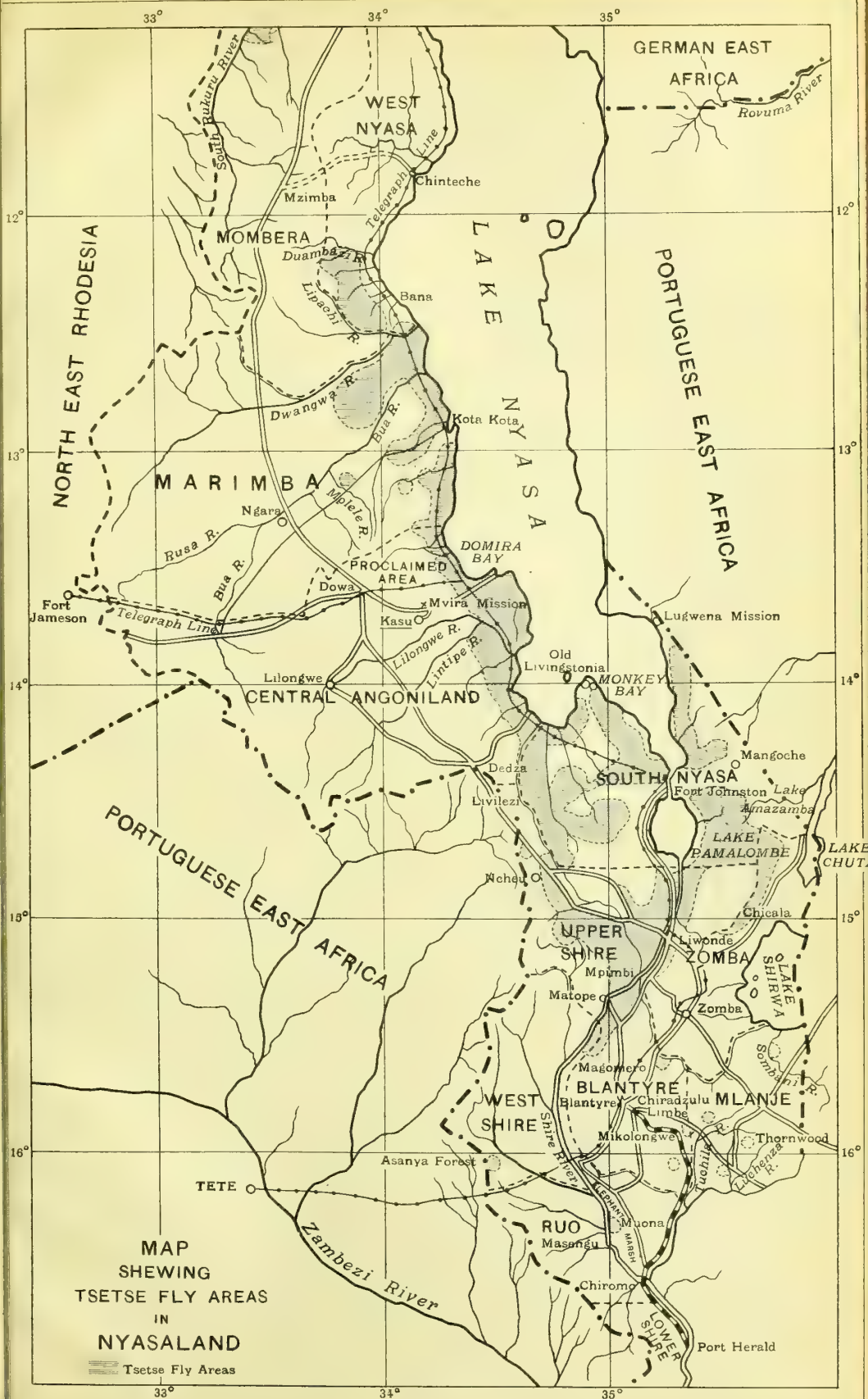
III.—LOWER SHIRE DISTRICT.

No species of tsetse fly has been found. No increase or decrease in the numbers of big game has occurred since 1909.

IV.—RUO DISTRICT.

Glossina brevipalpis occurs in two localities, at Masengeri and Muona, in the Elephant Marsh. In the latter locality three specimens of what were supposed to be *Glossina fusca* were captured.

The big game has decreased by at least one-half since 1909.



V.—WEST SHIRE DISTRICT.

Tsetse fly is unknown. There has been a distinct decrease in the big game since 1909.

In a further report, the Resident reports the finding of one *Glossina morsitans* in Asanya forest, about two hours to the west of Chagoma village.

VI.—BLANTYRE DISTRICT.

Blantyre Division and Chiradzulu Sub-District.—*Glossina morsitans* represents, as far as is known, the only species of tsetse fly found in the Blantyre Divisions. There is a well-known fly-area stretching across the Blantyre-Matope road from the Lirangwe drift northwards.

There are three other areas marked in the map where cattle are located and have suffered from trypanosomiasis, though no fly has been observed in these localities. These areas are as follows:—

1. *Magomero*.—This estate is situated in the Chiradzulu sub-district. In 1913 the trek oxen employed between Magomero and Limbe on the macadamised main road began to die in numbers from trypanosomiasis, though the cattle that had not left the estate remained healthy. It has not been proved where the animals became infected. No fly has been observed on the Magomero estate or on the road between the Namadzi river and Limbe.

2. *Mikalongwe*.—During the past three years there have been deaths from trypanosomiasis among the herd of cattle on the British Central Africa Company's estate in this locality, but no tsetse fly has been observed. The animals are supposed to have been bitten by tsetse on the grazing grounds between Mikalongwe and Mpezo. There are a few sable antelope in this locality, but no other species of game.

3. *Gotha*.—Cases of trypanosomiasis occurred in 1912 among the cattle on the Gotha estate of the African Lakes Corporation. The herd had hitherto been free from this disease. Large game is almost entirely absent, though wild pig, bushbuck, and duiker are present in some numbers. No tsetse fly has been found.

Big game has decreased in this locality since 1909. No tsetse fly has been observed in the Chiradzulu sub-district. Game has decreased throughout the Blantyre district.

VII.—MLANJE DISTRICT.

The late Dr. P. Wykesmith reported prior to the year 1909 that *Glossina morsitans* existed in three localities in the Mlanje district:

1. Near the Sombani river in the sphere of Chief Mtemanyama.
2. Near the Tuchila river by the Blantyre-Mlanje road crossing.
3. North-east of the Thornwood estate at the back of Mlanje mountain.

No other competent observers have, however, reported the existence of *Glossina morsitans* since the date of the late Dr. Wykesmith's report.

Mr. S. A. Neave, in the year 1912, proved the existence of *Glossina brevipalpis* in the neighbourhood of the Lichenya river about one and a half miles south of the Mlanje-Ruo main road, and considers that this species exists along the south-east side of Mlanje mountain.

There is no doubt that the big game has decreased in number since 1909 owing to the extensive clearing of scrub consequent on the increase of the native population and the opening of new estates by Europeans and the extensive hunting of game.

VIII.—ZOMBA DISTRICT.

(a) *Zomba Division*.—*Glossina morsitans* probably occurs in the Zomba Division at various times, owing to its being brought up by natives and game travelling from fly-areas in the Liwonde sub-district, but no definite belts have been discovered. One specimen of *Glossina morsitans* was captured at the house of Mr. E. A. Hooker on the Namadidi estate some five miles from Zomba. This planter lost practically his entire herd of cattle (about 200 to 300 head) from trypanosomiasis since 1909. Trek oxen employed on the Zomba-Blantyre road have also been infected with trypanosomiasis.

Big game has undoubtedly decreased since 1909.

(b) *Chikala Sub-District*.—*Glossina morsitans* is distributed throughout the Chikala sub-district, and appear to have increased since 1909. Big game is probably more plentiful than in 1909.

IX.—UPPER SHIRE DISTRICT.

(a) *Ncheu Division*.—*Glossina morsitans* has alone been observed and the fly-belts are reported not to have altered to any great extent during the last four years, and while several new belts have been observed since the 1909 report, it cannot be asserted that they were actually non-existent in 1909. No decrease of *Glossina morsitans* has been noticed in any of the localities in question.

There has been some decrease in big game during the last four years, but the game areas are practically the same, although the numbers of animals are not so large.

(b) *Liwonde Sub-District*.—The only species is *Glossina morsitans*, which is now found over about two-thirds of the sub-district. In numbers the fly varies largely from year to year, and from month to month. It is most plentiful in the well wooded country bordering the Shiré river, and appears in greatest number towards the end of the wet season. In one of the places where fly is plentiful game is particularly scarce, crossing the fly belt only at night to get to water.

The spread of the fly during the last four years has been considerable.

There has been a slight decrease in big game since 1909.

X.—SOUTH NYASA DISTRICT.

For the purpose of the report the district has been taken as being divided into two portions: east and west of Lake Nyasa and the Upper Shiré river.

(a) *Western Division*.—*Glossina morsitans* is found along the whole length of the Livelezi valley, and thence round the south-west arm of Lake Nyasa to the hills south of old Livingstonia, and has increased in number. Big game has always been plentiful in this locality, but no increase or decrease can be pointed to since 1909.

In the vicinity of Malembo the fly is less numerous than elsewhere in this belt, though with the exception of roan antelope other kinds of game, especially eland, are numerous.

At Monkey Bay, in addition to *Glossina morsitans* there are found *Glossina pallidipes* and *Glossina brevipalpis*. These species occur together in close proximity to the lake. With the exception of buffalo, big game is very numerous, and there has been an increase in game and fly alike.

In the neighbourhood of Fort Johnston *Glossina morsitans* is increasing.

Along the Fort Johnston-Liwonde road fly is scarce. On the hills immediately behind the road a dense belt extends coterminously with the higher ground, but game is not numerous.

(b) *Eastern Division*.—On the east of the lake, from latitude 13° 30' down to the Lugwena Mission, there is a total absence of fly. Elephant apart, big game has probably decreased since 1909.

At Mangoche no fly is found, and game, with the exception of klipspringer, is absent.

In the plains below Mangoche *Glossina morsitans* and game have both increased since 1909.

Along the east shore of the lake *Glossina morsitans* occurs in the valleys and not on the hills, and, like the big game, shows no increase since 1909.

General.—Regarding the district as a whole, *Glossina morsitans* has increased since 1909. The number of *Glossina pallidipes* and *Glossina brevipalpis* have remained stationary. Big game has increased in certain localities and decreased in others, with a net result equal to that obtaining in 1909.

XI.—DEDZA DISTRICT.

Glossina morsitans extends in large numbers over the whole of the lake shore area of the district, but is not found on the plateau. The fly belts extend from within a mile or two of the actual shore of the lake right up to and among the foot hills.

At various seasons of the year the difference in the number of tsetse flies encountered is very noticeable. They are most numerous at the end of the rainy season (April to May). From April to August the flies diminish steadily in number, and from thence onwards until the beginning of the rains there is no appreciable change. Early in November the number increases gradually, but does not attain its maximum until the following April. The habitat of the fly is, as a rule, a well-wooded area with thick undergrowth of from three to six feet in height. Open, treeless country is usually found to be free from fly. The presence of water is stated to offer no positive attraction.

The localities where game is most abundant coincide with those where *Glossina morsitans* is most numerous. It is reported that the number of tsetse fly in a certain area diminished after the game had been driven off to other localities by wild dogs.

No increase or decrease has taken place in either the tsetse-fly areas or the big game since 1909.

XII.—LILONGWE DISTRICT.

The tsetse-fly area comprises the whole of the country below a level of 2,500 feet, with the exception of a few miles of plain and timberless country in the vicinity of Domira Bay. There has been no alteration in the area since 1909. Big game do not appear to have increased within this area, but an increase is noticeable in the remaining portion of the district.

XIII.—MARIMBA DISTRICT.

(a) *Kota-Kota Division*.—Both *Glossina morsitans* and *Glossina brevipalpis* exist. The areas infected by *Glossina morsitans* have not increased to any appreciable extent since 1909, as with one exception, that of a belt on the Mpelele river, the belts remain the same. The existence of *Glossina brevipalpis* has been proved since 1909, but this species of tsetse fly may have been present, though not reported, in 1909. Tsetse flies are reported to vary greatly in number during certain years.

The numbers of big game have slightly decreased since 1909, especially hippopotami, eland, kudu, waterbuck, and hartebeeste, but buffalo, elephant, and rhinoceri have slightly increased.

(b) *Ngara Sub-District*.—*Glossina morsitans* only is found, and is on the increase.

Big game is steadily increasing.

XIV.—WEST NYASA DISTRICT.

The existing tsetse fly area lies in the extreme southern part of this district and stretches from about three miles north of the Doambai river to the Dwangwa and Limpachi rivers. On the eastern side of this belt tsetse have not been found within three miles of

the lake shore, north of Bana, nor within seven miles of the lake shore south of Bana. On the west side the belt extends nearly up to the boundary of the West Nyasa and Mombera districts.

The belt consists entirely of *Glossina morsitans*, though two specimens of *Glossina pallidipes* have been captured on the Dwangwa river.

Glossina brevipalpis (probably the sub-species *longipennis*) is reported to have been found on the Rukuru river.

The tsetse fly area has neither increased nor decreased since 1909.

With regard to game, both eland and sable have increased within the tsetse fly area.

Game of all kinds, except pigs and duiker, are extremely scarce throughout the district save in this fly area. Reedbuck, hartebeeste, and zebra have decreased. Waterbuck have increased, and wild pigs also greatly increased. The small herd of buffalo on the Limpasa river is distinctly larger since 1909.

XV.—MOMBERA DISTRICT.

Glossina morsitans only has been observed, and is confined to a very small area.

There has been no increase or decrease in the fly since 1909.

With regard to big game, buffalo have increased considerably, and eland also, but to a lesser extent. Other big game, especially zebra, have decreased.

XVI.—NORTH NYASA DISTRICT.

Glossina brevipalpis has been found on the Lufira river eight miles north of Karonga, also on the lower course of the Songwe river. One observer states that he noticed tsetse fly (supposed to be *Glossina morsitans*) in two localities, firstly, between Kaseka's village on the Mwasisi river and the South Rukuru river in Chikula-mayemba's country, and secondly, in the tree country to the north of the Vwaza marsh.

Big game appear to show no decrease and is plentiful in many parts of the district.

(Signed) A. M. TURNBULL,

Acting Secretary to the Administration.

Zomba, 10/11/13.

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THIRD, FOURTH AND FIFTH REPORTS

ON

THE BIONOMICS OF
GLOSSINA PALPALIS
ON LAKE VICTORIA.

BY

G. D. HALE CARPENTER, M.B.E.,
D.M., B CH. (Oxon), F.E.S., F.Z.S.
Uganda Medical Service.





ROUTE OF JOURNEY
Jan Feb March
1914

(Islands examined in 1912)
on Feb
"Islands" examined in 1914

Based upon chart of Commander A. H. S. P. H.

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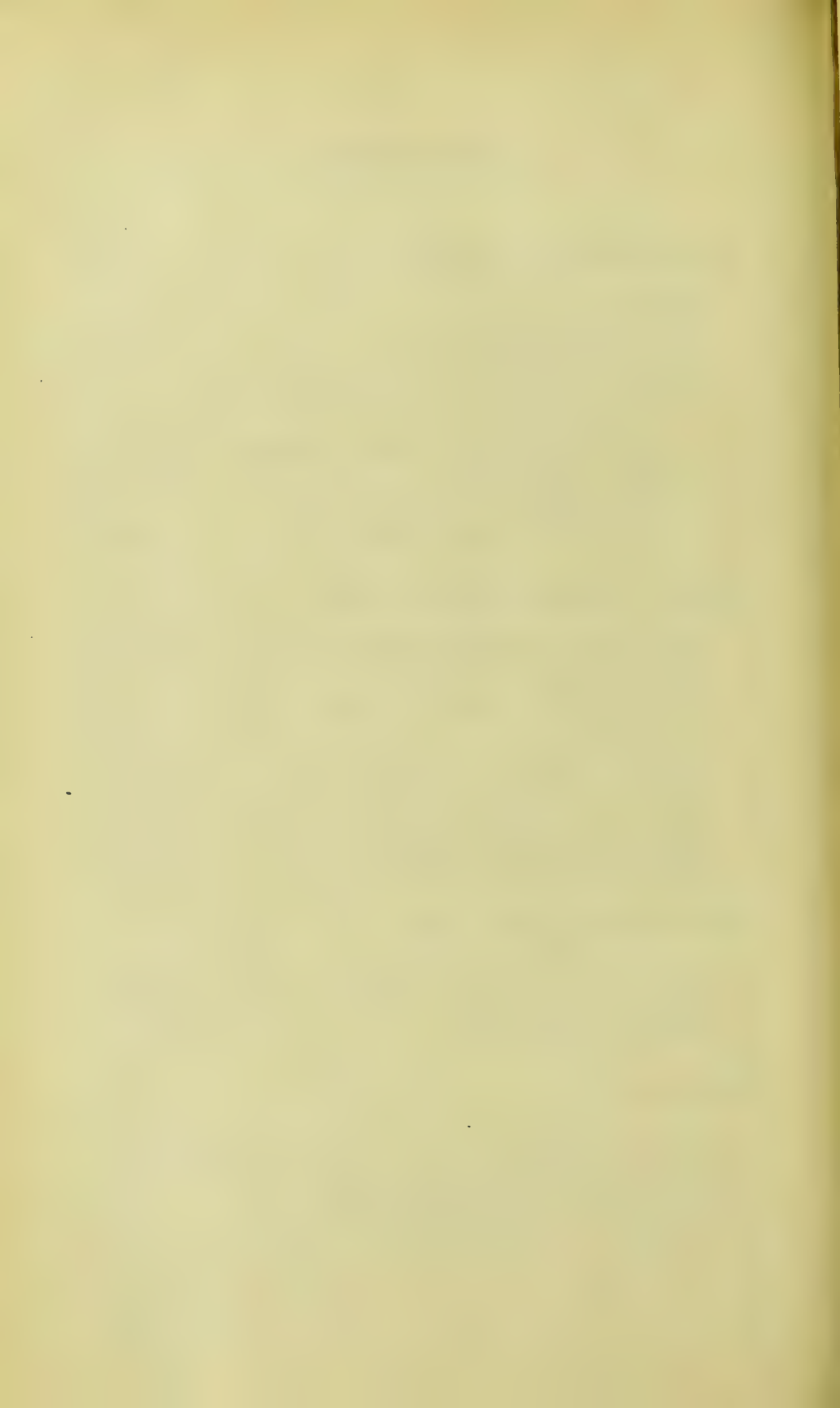
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THIRD REPORT ON THE BIONOMICS OF *GLOSSINA PALPALIS*.

ON SHORES OF L. VICTORIA.

During January, February, and March, 1914, a tour was made among the chain of islands lying parallel to the coast of Lake Victoria between Entebbe and Jinja, at the suggestion of and in company with Mr. W. F. Fiske. The object of the tour was to endeavour, by means of comparison and elimination, to ascertain what were the factors causing *Glossina* to vary in abundance on the different islands. I wish to make the fullest acknowledgment of the help received from Mr. Fiske during the tour: much has been brought to my notice by him, the significance of which I should otherwise have overlooked.

The following are the conclusions at which I have arrived as a result of the tour:—

1. To support fly, an island must be of a certain minimum size, however suitable it may be in other ways.
2. An island must have a certain minimum amount of good shade in proportion to its area. So long as there is good shade near the water its absence elsewhere does not act unfavourably to the fly. Provided there is a certain amount of good shade the fly may extend its range to parts where there is such poor shade that it would be insufficient if it were all that the fly had to depend upon. Vegetation which grows too densely is not associated with the presence of great numbers of the fly. This is well known in the case of Papyrus.
3. There is much to be said for the hypothesis put forward by Mr. Fiske, that the percentage of females caught by boys is an indication of the accessibility of food supply. On small islands from which buck are absent reptilian blood is very much preferred by the fly to the blood of hippopotami, bats, or otters, which are the only sources of mammalian blood on such islands.
4. It seems probable that variations in the amount of food (blood) available will influence, through the rate of reproduction, the numbers of the adult fly.
5. Shade produced by fresh green growth, as of a certain aromatic herb, is at once made use of by the fly for larviposition. Hence pupæ may be found at different times in different places according to the local condition of the vegetation. At other points the shade is always uniform and such localities are always suitable for pupæ. Pupæ are readily killed by diminution of the density of shade.
6. The fly can exist in certain numbers in localities where there are no localized breeding grounds. For greater abundance, breeding grounds are necessary. It is therefore obvious that clearing such breeding grounds of their shade-producing vegetation will not wholly banish the fly. These breeding grounds may be completely invisible from the water.

- * 7. Hymenopterous parasites (a new species of Chalcid described as *Syntomosphyrum glossinæ* in the Bulletin of Entomological Research, Vol. V., Part IV., p. 365) have been reared from pupæ but they are very scarce.
- 8. On certain islands, where conditions appeared generally favourable for the existence of the fly in large numbers, abundance of large orb-web-spinning spiders may account for the small numbers of fly that were actually found. This must at present be regarded as not proven.

In order that the report may be intelligible, a map is attached (frontispiece), together with an account of each island visited, and notes on its peculiar features.

Particular notice was taken of the Lepidopterous fauna, since it was desirable to ascertain if *Glossina* was especially abundant here or there because the conditions were particularly favourable to insect life, as might be indicated by abundance of butterflies. It will be seen, however, that no correlation was found between numbers of butterflies and of *Glossina*.

On arrival at a fresh island one, at least, of us always circumnavigated it in the canoe, endeavouring to estimate the abundance of animals that might provide food for the fly. The coast was always further explored on foot, and it was found that much more could be learnt in this way of the suitability of the coast for breeding grounds than by inspection from the water.

Flies were caught at various points around the island, wherever it seemed likely that they would be present; and search was similarly made for pupæ. Many islands were too large to be thoroughly examined in the time at our disposal; the smaller ones by repeated visits were fairly thoroughly investigated. The first to be visited was Bulago (see map) on which we camped from January 12th—24th. From it were visited Kizima, Tavu, and Resuvu.

On January 24th camp was moved to the E. end of Nsadzi, from which Kimmi and Ngamba were visited.

We moved again on February 5th to the E. end of the large island Kome, visiting from there Sanga, Lula, Kisigalla, Dwanga Mkuru, Dwanga Mto, and Dwasendwe, also part of the S.E. coast of Damba.

Camp was moved to Kerenge on February 13th, and we investigated *en route* the two minute islets Maungwe and Enkusa. From Kerenge were visited Isentwa, Ziro, Lukalu, and Kiuwa.

We moved to the south shore of Wema on February 20th, and on the way explored the small isle of Zigunga and parts of Kibibi. From Wema we worked on Marida, Sege, Waitwe, and Wavuziwa, and visited Yempata and Sindiro but had not opportunity to work them thoroughly.

This was as far as we could go, and on March 2nd we returned to the former camp on Kerenge, visiting Kibibi again and going again to Kiuwa from the camp.

On March 4th we had to hurriedly return nearer to Entebbe as food was running short, and we camped on the N.W. point of Damba. From here I visited Dyavodemi and its neighbour

Masovwi, and the several little islets Mtiomu, Tokwi, and Kawaga off the point of Damba.

On March 7th we returned to Bulago, and re-investigated it together with Tavu and Kizima; and, having moved to Nsadzi again on March 16th, we revisited Kimmi and Ngamba and I went to Rumfua off the S. shore of Kome.

On March 22nd we returned to Entebbe.

DESCRIPTION OF THE ISLANDS VISITED.

1.—BULAGO.

The centre of Bulago is occupied by open grassland, and two rounded hills rise to a height of a little over 200 feet. One at the W. spur has very steep sides where it abuts on the water; the other, at about the middle of the island, descends more gradually.

There is dense forest on the narrow northern peninsula, where were the plantations of former days; also on the S. aspect of each of the hills. Along the shore of the N. bay is a belt of brush and forest, with a good deal of the plant known to the Baganda as "*E'tungulu*." This will be frequently alluded to. It is, I believe, of the family *Anonaceæ*, and has straight, stiff, but not woody, stems rising individually from the ground often to a height of 8-10 feet. The large, smooth, and shiny leaves come off alternately from these stems, which being close together thus throw very good shade. The roots ramify just below the surface. Clusters of shining red fruits, containing round, black seeds in acid pulp, are borne on short stems rising straight from the ground. There appear to be two species of *E'tungulu*, one having the flowers red, while in the other they are white.

To continue the description of the island. The S.W. shore has, between the bases of the two hills, a strip of fine white sand on which grow thickets of cane and *E'tungulu* with a bush, or small tree known as "*Omuwuvumye*." This is a papilionaceous tree with finely divided leaves (a species of *Smithia*) and is constantly found near the water. Although it does not of itself throw much shade, yet it helps to form thickets over which shade producing creepers can entwine.

The S.E. point of Bulago is formed entirely of fine white sand with thickets formed of cane and the species above mentioned and many ambatches at the water's edge.

Along the rocky E. coast there is little but cane and low bushes and a fringe of rushes in the water.

Hippopotami frequent the N. part of the island, and appear often to visit the S. point, where otters have been seen. No evidence was obtained of the presence of buck. Bats abound in the bush. The island is not greatly frequented by the cormorants, darters, herons and egrets, which usually abound. The Hagedash ibis was quite abundant however.

Flocks of bee-eaters and swallows come and go, and the forests are well frequented by the usual insectivorous birds whose notes render them obvious (fly catchers, coucals, etc.).

As regards reptiles, the sandy S.E. point seems particularly well suited for crocodiles, which are often seen basking there.

They also frequent the northern point, on which is a sandy area along the otherwise rocky western aspect, and here a nest was found on March 14th containing 64 eggs, whose embryos were nearly ready to hatch.

The huge lizard (*Varanus niloticus*) known to the Baganda as "Enswa-swa" is, relatively to other islands, not very plentiful on Bulago, and seems to prefer the more forested northern part of the island.

A red, spiny-backed, orb-spinning spider of medium size was found more abundantly on Bulago than on any other island visited. Only a single specimen was seen of the enormous spider (? *Nephila*) to be described as so abundant on other islands. Lepidoptera were numerous. A total of 61 specimens was noted on the first visit, and in March 13 more were added. Acraeines and Lycaenids were abundant in species as well as individuals, 13 of the former and 27 of the latter being noted. As regards Hymenoptera, *Bembex forcipata*, *Handl.*, abounded on the sandy S.E. point.

2.—KIZIMA.

This small island, lying to the W. of Bulago, has in some respects a resemblance to it. It consists of a grass covered hill rising steeply from the water on the W. side, but less steeply to the E., so that on this side there is a narrow fringe of bush along the shore, mostly of the species known to the Baganda as "*Oluzibaziba*." It is a Euphorbiaceous bush (*Alchornea cordata*, *Benth.*), which, having large leaves like poplar, and growing in thickets, produces very excellent shade.

On the W. side of Kizima a few fig trees grow on the rocky shore. There is one small bay with fine white sand in which grow the Muvuvumye trees above mentioned, throwing light shade. There is no forest growth on the isle, and only one or two large trees at the S. end of the island.

Although Hippopotami were not seen on, or near, the island, yet when we visited it several of their sleeping places were seen there, and on a recent visit a young one was found on the island. A feature of Kizima is the great number of rodents living among the tussocks of thick grass which clothe the hill. Bats were observed among the thickets of Luzibaziba on the E. coast.

On our first visit to the island two crocodiles were seen on the rocks of the W. coast, and subsequent visits have shown that they certainly, at the present time, constantly frequent the island. *Varanus* is fairly abundant. Although only two were actually seen, yet their basking grounds on the rocks were very numerous.

One small snake, very beautifully procryptic, was found coiled among the twigs of a bush, having very recently fed. This species has not been noted elsewhere, but this is probably accounted for by the perfection of its concealment.

Birds around the shore were numerous. Cormorants, darters and egrets were on the rocks, together with a Sacred Ibis, which has been always seen in the same place at every subsequent visit. This bird is extremely scarce, and was not met with on any of the other islands except Nsadzi. Small birds among the bushes were scarce, but two common flycatchers were noted, and a third species which has a particularly sweet song reminiscent of a nightingale, and seems to be especially characteristic of quite small islands, was also found to be present. It is brownish grey with white

throat and belly, and will be alluded to as the "Nightingale fly-catcher."

Lepidoptera were extremely scarce on Kizima; at the time of our first visit only four species were noted, of which one at least may have flown across from elsewhere. Three additional species have been seen on subsequent visits.

An interesting fact is that no large termite hills were to be seen on this island, but no other peculiarity of insect fauna was noted.

3.—TAVU.

Tavu is covered with forest and jungle save for small open grassy spaces close to the shore on the E. and W. sides, and for marshy areas on which grow lush grass and water loving bushes along the S. shore. This type of shore is alluded to hereafter as "grazing ground," for hippopotami are especially addicted to such localities, which are always intersected by their tracks. At the northern end of the island is an extension formed mainly by ambatch growing either in; or just out of the water, and there is also much ambatch along the eastern shore; the W. shore presents, in most places, a wall of E'tungulu and bush, there is no open sandy beach to be seen from the water, the shore line being either rocky or marshy.

Hippopotami are always to be seen in the neighbourhood of the shore, and they have regular paths through the old plantations occupying the flat centre of the island, where bats, as usual, may be seen hanging.

More crocodiles were seen around the coast of Tavu than on any other island visited, nine having been counted when going round it in a canoe. An old nesting place, marked by the empty shells, was found on an old sandbank on the W. side of the island, thrown up when the lake was at its former higher level.

Varanus was not at all plentiful on Tavu, only two having been seen, and their traces were not at all in evidence. A large black snake—usually met with on most islands—seemed particularly abundant on Tavu, and on one occasion a small python was seen swimming some thirty yards away from the shore.

Birds abounded both around the shore and in the thick jungle, the characteristic notes of many coucals, flycatchers, bee eaters, etc., being heard, and wagtails being plentiful on the open grassy space on the W. side. In short, there was evidence that Tavu was eminently suitable for abundance of insect life. At one point on the W. coast spiders' webs were noted covering a small dead bush with a glistening white mass, but elsewhere spiders were not particularly abundant.

Chrysops brucei was noted to be more abundant than on any other island visited during the tour.

4.—RESUVU.

Lying about half a mile to the S.E. of Tavu is a small sand spit, most of which is below the surface of the water at all times, and is covered with a thick growth of ambatch bushes.

Its S. end, when visited during the dry season, was above the water, forming a stretch of sand about fifty yards long and twenty broad, fairly well covered with Muvuvumye trees and creepers. Numbers of cormorants and darters nest on Resuvu, stone curlews are seen on the exposed sand, and hippopotami, crocodiles, and *Varanus* all visit the island. Since all the conditions seemed quite

suitable for the presence of *Glossina*, I was at a loss to account for its absence in January when Resuvu was first visited. Subsequent visits during May, when owing to heavy rains the lake is at a higher level, show that the sand which was exposed in January was in May almost covered by the water, so that it was not dry enough for breeding purposes.

5.—NSADZI.

The greater part of Nsadzi consists of a grass covered ridge, rising fairly steeply to a height of about 250 feet at the middle. About one-fifth of the island, at the eastern extremity, is flat, and sharply delimited by a small escarpment. This part is covered with dense forest, which is quite cut off from the other forested parts of the island by bare, rocky shore, and the grassy heights in the centre of the island. About at the middle of the north shore there is a patch of forest and jungle which extends to the water; this marks the site of the plantations of former days.

On the south shore is a shallow bay the beach of which is of pure white sand; behind it rises a cliff at the foot of which grows a belt of dense forest. These conditions form a typical "Fly beach." Between this forest and the forest-clad eastern end of the island the south shore is bare of trees save for two isolated patches of bush of small size. Westwards there are one or two more small patches of bush, but the south shore generally is very bare and rocky. There is a very narrow line of bushes (Kinsambwe) around the western end of the island which is continued to meet the forest on the north shore.

The grassy uplands are exposed, and bare of forest save for a small patch about in the centre of the island. The coast line generally is rocky, and only on the south aspect of the eastern flat end is it marshy in places. Besides the sandy bay mentioned above there are two sandy stretches of coast on the north shore, but they have very little shade. Hippopotami are always to be seen off some part of the coast of the island.

There are now frequently to be seen tracks of the Sitatunga Antelope on the uplands, though curiously enough the tracks have only once been seen on the sands of the south bay. Presumably the animals spend the daytime in the forest of the eastern end. I understand that in former days when Nsadzi was inhabited these buck were never seen on the island. The tracks of otters may often be seen on the sand; and bats as usual frequent the jungle.

Neither crocodiles nor *Varanus* can be said to be abundant on Nsadzi, although a small crocodile may almost always be seen on the shore of the south bay, and *Varanus* tracks are to be seen there. Puff-adders may be met with on the grassland.

As regards birds, the forest abounds with coucals, bulbuls, and flycatchers; and weaver birds are common along the shore. Cormorants and darters are not very abundant, owing probably to the lack of bushes along the water's edge.

On the sands of the S. bay may always be seen a pair of the Egyptian geese—not by any means generally met with; and on the rocks off that coast the Sacred Ibis may be seen—a rare bird among the islands.

With regard to Lepidoptera, it was impossible during the time at our disposal to estimate the number of butterflies, but there were a considerable number of species. One *Lycaenid* was

taken, which, though common on the mainland, was not met with on any other of the islands visited.

A striking feature when we first visited the island in January was the multitude of larvæ of a small Noctuid moth on the open grassland. The tussocks of grass had been recently fired, and were now sprouting again, and over many square yards the young blades had been entirely devoured by these larvæ which could be counted by dozens on every large tussock.

Another species of Noctuid seemed particularly characteristic of Nsadzi, a large, black species with eye-like markings and white stripes on the wings, which takes refuge during the day in the deep holes made where the light soil of the grass land, often undermined by termites, has given way under the feet of hippopotami. These holes, perhaps twelve inches deep, were often found to shelter several of these moths at once.

A species of *Bembex* (*B. forcipata*) was noted abundantly on the sandy shore of the south bay. This was recorded in my first report, and was first noted in March, 1911. It was not seen in January on our first visit of the present year, but appeared in numbers in March.

There was a small species of *Simulium* on Nsadzi, which bit freely at sunrise and sunset. The flat rocks shelving gradually into the water at the neighbourhood of the camp seemed a particularly suitable locality for this pest.

6.—KIMMI.

This flat island presents a great variety of conditions. A great part of it is occupied by a patch of forest, the trees of which are of remarkably few species. *Chrysophyllum* (*Sapotaceae*) and one other predominating. The north and south shores are of the type which has been called "grazing ground" and are much frequented by hippopotami. There are bushes scattered about amidst the lush grass, growing practically in the water when the lake is high, together with patches and belts of the E'tungulu plant. At the north-west corner of the island and on the south-east side, are thickets of the Kinsambwe and Luzibaziba bushes.

On the west side is an open grassy space between the central forest and the thin fringe of bush along the shore; on the east side is a large open sandy area, on which grass grows thinly, bordered narrowly at the water's edge with cane and E'tungulu and a few bushes.

The character of the shore varies greatly. The S.W. point is rocky with patches of sand interspersed. On its south aspect there are several large caves at the foot of a rocky escarpment, in front of which is low-lying sandy shore. Passing eastwards along the south shore one comes to the grazing grounds above described, where the shore is fringed with ambatch trees, and then to a sandy beach, fringed with cane, etc., at the edge of the open grass area.

Continuing round the coast one comes to the marshy northern grazing ground, behind which is a sand bank left when the lake was formerly at a higher level, and then to the rocky north-west point, amongst the rocks of which are areas of sand. The western coast is rocky, with sand at the back of the rocks.

Hippotami are very attached to Kimmi: a herd of five may constantly be seen off the south-west point, and their sleeping places in the forest are very conspicuous. No other mammals

save the usual bats, many of which frequent the caves, were noticed on this island.

No crocodiles were seen during our many visits to Kimmi, but later, on April 7th, I saw a small one on the rocks off the south shore. But their old resting places, marked by fragments of eggshells, were found on the N.W. point and on the north shore.

Varanus also was very scarce, which was the more remarkable because the open sandy grassland on the east side was, in places, riddled with holes dug by them, presumably in search of a suitable site for their eggs, of which empty shells were found there. We, personally, never saw more than one while on the island.

Snakes were not uncommon, several of the large black species and of the green grass-loving species having been met with. Birds abounded on the island. The ambatch fringed portions of the shore were frequented by numbers of the usual cormorants, egrets, etc., and flycatchers and coucals were abundant in the forest. The "nightingale flycatcher" was heard on Kimmi, and a pair of grey plantain eaters (which may constantly be seen feeding on ambatch blossoms) frequented the eastern grazing ground. These latter birds were not met with on other small islands.

Of Lepidoptera, forty species were noted at first, and one more on subsequent visits. Lycaenids were in rather large proportion, twelve species having been recorded, only four Nymphalines, and seven Acraeines. Nothing particularly characteristic was noted in the Arthropod fauna.

7.—NGAMBA.

This island, quite close to Kimmi and of about the same size, offers less variety of conditions. It rises higher in the centre, the summit of the small hill, perhaps 180 feet high, being crowned with bracken, while elsewhere the island is covered with forest which contains a much greater variety of vegetation than that on Kimmi. This forest comes everywhere to the water's edge, save on the eastern side at the head of a large bay, where there is a stretch of low, marshy, grazing ground of the usual type. On the north shore the former beach appears as a bank of coarse brown sand, now some yards back from the water, and at the northern end of the western shore, and at the north-east point the same may also be seen, but elsewhere the shore is formed of unbroken rock, or of boulders, or large pebbles. There is a long shingle beach on the south shore, forming a typical "fly-beach." At the south-west point there is a good deal of bare rock along the coast, the forest here not coming quite so close to the water.

Hippopotami do not frequent Ngamba to such an extent as they do Kimmi—though one may often be found off the N.W. point. It seems probable that the Sitatunga at least visits the island (these buck could easily swim across the narrow channel from Kome), for the ends of the branches of Luzibaziba bushes were found to have had the leaves eaten off in the manner characteristic of this antelope. But none were met with, nor were any other traces seen.

Otters are fairly abundant on the rocky eastern shore of Ngamba, and a mongoose was met with in the forest, which has not been seen on the other small islands visited.

An enormous congregation of the large "Flying fox" fruit bats was seen on our second visit in March among some tall creeper

covered trees in the forest. Such a congregation was not elsewhere met with among these islands. But in 1913 at the same time of year, I found one on Bunyama, in the Sesse Archipelago.

Other, insectivorous, bats are common as usual in the forest. Crocodiles are much more numerous on Ngamba than on Kimmi, indeed Ngamba comes only second to Tavu on the list (see Table I.). Six were seen when circumnavigating the island, and two places found where eggs had been laid, one on the N.E. and one on the N.W. point.

Varanus is also much more common than on Kimmi. Three were seen on the first visit, and five, at least, on the return visit. The large, black snake is quite abundant. There is nothing worthy of special notice in the avian fauna.

Lepidoptera were not so numerous as was expected from the abundance and variety of vegetation, only twenty-two species having been noted. A species of Psychid making smooth silken cases, covered with debris derived from bark, was extremely abundant on tree trunks in the forest. Nests of the tree ant, *Oecophylla smaragdina* were sparsely seen, they are not met with on all the islands. None of the big spiders so abundant elsewhere were met with.

8.—SANGA.

The little isle of Sanga, off the eastern spur of Kome, has a very interesting fauna and varied flora. The shore line is rocky everywhere except for one very small area where is a little light sandy soil; and the surface of the island generally is formed of rocks, amongst which grow ferns. Sanga is covered everywhere with forest, but there is remarkably little of the tangled bush often met with on these forested islands. No Hippopotami were seen, but they evidently frequent Sanga, and bats live in the forest. One young crocodile and two *Varanus* lizards were met with during our visit.

Birds were remarkably scarce on the islands, and the silence in the trees were very noticeable. No flycatchers, bee-eaters, coucals, or warblers were seen on the first visit, when weavers, parrots, and pigeons were the only birds noticed, and on a second visit one Paradise flycatcher was heard. This absence of bird life seemed to be correlated with abundance of predaceous arthropods..

Huge spiders and *Mantidæ* were very abundant indeed, and the webs of the spiders formed great sheets stretching from bush to bush. Lepidoptera were very numerous for an island of such small size, for thirty-five species of butterflies were noted. The chief peculiarity was that *Hesperidae* outnumbered any other group, eleven species having been recorded; Lycaenids came next with ten. A very handsome large Hesperid of metallic peacock blue and green tints (*Rhopalocampta chalybe*) was abundant, and I have never seen it elsewhere more than one at a time, whereas on Sanga several might be seen dashing about together, making a wonderful spectacle. Only three species of Acraeines were seen, and all were scarce. The Nymphalines included such forest genera as *Pseudacraea*, *Salamis*, and *Euralia*.

A certain shrub, growing abundantly, was in many places quite defoliated by the large numbers of larvae of a species of the Geometrid genus *Aletis*, which were quite a feature of the fauna of Sanga.

9.—LULA.

The islet of Lula, adjoining Sanga, presented considerable points of difference from it. It is a rocky ridge, covered all over with very dense thickets of *Luzibaziba* bushes, and with no forest trees. Hippopotami do not seem to visit it, but there were many basking places of crocodiles, and a *Varanus* was seen. Bats were seen among the bushes.

As regards birds, a small black and white fly-catcher was abundant—yet it did not occur on the neighbouring Sanga. Weavers were very numerous, and cormorants on bushes overhanging the water at one end.

The large spiders, and *Mantidae*, notable on Sanga, were absent from Lula. The butterflies also presented interesting differences for out of fifteen species recorded, seven were *Acraeines*, and only one *Hesperid* was noted, with four *Nymphalines*, and three *Lycaenids*.

10.—KISIGALLA.

This islet is of about the same size as Sanga, and like it, rocky, and well covered with trees and ferny undergrowth, the trees being of numerous species. It differs from Sanga by having on one side a flat piece of shore where is a bed of *E'tungulu* growing in sand. But there is no open sandy beach.

It is well patronised by Hippopotami, which sleep there, and the usual bats were seen. No crocodiles or *Varanus* were seen on Kisigalla.

Birds were more numerous than on Sanga, the Paradise fly-catcher being not uncommon; weavers, sunbirds, and pigeons abounding, and the red and blue plaintain eater being seen, though the latter had probably come over from Kome.

Large spiders were plentiful, though not so abundant as on Sanga. An unusual feature was the great numbers of a large black *Scoliid* that were flying about close to the surface of the ground. As regards *Lepidoptera* the most striking feature was the very large number of *Lasiocampid* and *Eupterotid* cocoons on the fig trees. Of butterflies, twenty-two species were noted, of which eight were *Lycaenids*, that being the greatest number seen of any one group; an interesting species was the forest-loving *Mimacraea poultoni*.

A peculiar feature of the fauna of Kisigalla was the abundance of a large cream coloured slug, which, though generally distributed, has never been elsewhere met with in such numbers.

11.—DWANGA MKURU.

This island has a central grass-topped ridge sloping steeply to the water on the east but forming a cliff on the west. The slopes of the east side are clothed with thin forest, the trees being small and apparently suffering from exposure to wind; amongst them are open spaces clothed with thin grass. The west side of the island at the foot of the cliff where there is shelter has very dense and rich forest growth, and was at one time under cultivation. The shore of Dwanga Mkuru is rocky, with here and there a small shingle beach; there is no sand to be seen.

Everywhere are dense masses of fern, but except at the N. end no *E'tungulu* or cane thickets. There are thick beds of a plant

with blue flowers which I believe to be a *Salvia*; it has large, broad leaves and grows to a height of nearly six feet, and thus throws dense shade. It is especially associated with rocky shores.

Hippopotami frequent the old plantations on the island, and bats as usual hang among the thick bushes. No crocodiles were seen, and only two *Varanus*, which is a small number for an island of this size.

Great scarcity of insectivorous birds was a feature of Dwanga Mkuru. The Paradise flycatcher was not once heard, and no coucals were noted, and the small birds usually seen in the undergrowth of forests were apparently absent. This scarcity of birds was associated, as on Sanga, with great abundance of huge spiders and *Mantidae*.

Lepidoptera were strikingly numerous on Dwanga Mkuru, both in species and individuals. Sixty-five species of butterflies were noted. Nymphalines were very well represented, *Salamis* being extraordinarily abundant. Acraeines were relatively scarce, and *Hesperidae* were plentiful, but the beautiful blue species so abundant on the neighbouring Sanga, was absent from Dwanga Mkuru.

12.—DWANGA MTO.

Like its larger neighbour this has a high ridge in the centre, which is covered with old plantations forming comparatively open jungle. Along the coast is forest and bush, but, as on Dwanga Mkuru, this appears to suffer from exposure on the E. side where it is thin and poor with open spaces.

The coast line is mainly rocky with ferns and *Salvia*, but the south face has a flat, low pebbly shore with rushes and water-loving bushes. On the west shore was growing abundantly a species of bush not previously noted, but subsequently found also on Dwasendwe and the west end of Kome.

Hippopotami visit the old plantations, where numbers of bats live. No crocodiles were seen, and only one *Varanus*.

Birds were not so remarkably scarce as on Dwanga Mkuru; and bulbuls were very noticeably abundant, also sun-birds (one of which was found firmly entangled in the web of one of the huge spiders which were very plentiful). The Paradise flycatcher, which was absent from the larger Dwanga, was noted on Dwanga Mto.

Butterflies were not quite so plentiful as on Dwanga Mkuru; the most striking feature was the almost complete absence of Acraeines. I only saw one specimen, and Mr. Fiske and I together could only record two species.

Lycaenids were most numerous, sixteen out of a total of fifty-seven having been counted. There were fifteen *Hesperidae*, a large species of *Rhopalocampa* (*R. forestan*?) being much more common than in any other locality visited.

Nymphalines were more numerous, in proportion, than on many islands, thirteen species having been noted. *Salamis* was particularly abundant.

Larvæ of a species of Psychid moth were extraordinarily abundant on the trunks of some trees, very many of their long tubular cases often being seen affixed to one tree on its sheltered aspect.

Of *Orthoptera*, *Mantidae* were common, but a large Acridian was unusually abundant and quite a feature of the fauna.

13.—DWASENDWE.

This island is covered with forest, and in its centre with old plantations overwhelmed with jungle.

The coast is rocky with thick beds of ferns and the *Salvia* previously described, save for a piece of the W. side where the shore is flatter and in places sandy, with ambatch in the water.

Hippopotami much frequent the old plantations, and bats live there. No crocodiles were seen, nor was *Varanus* met with, although at least one basking place was seen. The big spiders were extremely abundant.

Of butterflies, forty-four species were noted. The most remarkable feature was the almost complete absence of Acraeines. Mr. Fiske and I between us only saw two individuals, belonging to two different species. There were fifteen *Lycaenidae*, one of which was only met with here and on Dwanga Mto, and I have never seen it before in Uganda.

The next largest group was *Hesperidae*, of which twelve were counted, Nymphalines coming next in order with five species.

Dwasendwe island, like Sanga, was very noteworthy for the great abundance of Geometrid larvæ, a species of *Aletis*, which in many places had completely killed the shrubs owing to the numbers feeding on them (cp. Sanga).

14.—DAMBA.

We had not sufficient time to investigate more of Damba Isle than that part of the S.E. coast lying west of the equator. Damba is a flat forest covered island, and the forest comes to the shore everywhere. In places there is a little foreshore; at one part this is of shingle, backed by the sand banks thrown up in former days. Along some parts of the shore there are Papyrus and rushes; other parts are rocky or sandy, but everywhere the forest comes close to the water.

In proportion to the size of the island there were remarkably few crocodiles or *Varanus* along that part of the shore which we explored. Hippopotami have their regular tracks through the forest leading to landing places.

It was impossible to get any estimate of the butterfly fauna of Damba, but I know, from nine months' residence on the island in 1911, that the forest has a rich fauna. Birds of course abound in the untouched forest. The big spider was only met with on Damba, here and there, and not in any quantity anywhere.

15.—MAUNGWE.

A mere crest of rock, measuring about 100×30 yards, and covered thickly with ferns, an aromatic herb, and *Ipomaea* creeper. Along the rocky shore scattered fig trees and few bushes. No crocodiles or *Varanus* were met with on this island; small brown lizards were very abundant.

Swallows and weaver birds were conspicuously abundant, and the flycatcher with nightingale-like song was abundant also. Although no species of Lepidoptera were seen, yet a species of *Belonogaster* wasp, which feeds its larvæ on caterpillars, was found on the island. A single specimen of the huge spider was seen.

16.—ENKUSA.

This is a spit of spongy brown rock about a hundred yards long, and disintegrated at one end to make a pebble ridge. At the other end is a dense growth of ambatch bushes. An astonishing variety of plants grow on the ridge, and Muvuvumye shrubs furnish fair shade.

One *Varanus* was found on the island. Weaver birds were plentiful among the ambatches.

Small as is the island, one species of feebly flying Lycaenid butterfly lives on it.

17.—KERENGE.

Kerenge is one of the medium-sized islands on which there is a considerable amount of open grassland, with patches of forest and plantations over-run by jungle. There is no sandy fly-beach, the shore for the most part being rocky, or with belts of cane and E'tungulu growing in light soil. There are some sandy areas a little way back from the present lake margin.

Hippopotami as usual frequent the island, and *Varanus* was met with, but not abundantly.

The large spiders were extraordinarily abundant in the old plantations.

Forty-five species of butterflies were counted, one-third of which were *Lycaenidae*. Thirteen species of *Hesperidae*, but only three *Acraeines* were recorded, the latter being remarkably scarce.

18.—ZIRO.

One of the islands having large central grassy spaces and forest along part of the shore, with old plantations now succumbing to jungle. The forest and jungle are mostly on the north and west, with belts of cane and E'tungulu at the edge of the water; the south and east coasts are bare, rocky, and of very irregular outline, with sparse cane and ambatch.

Three Hippopotami were seen in the water off the north-east part of the island, and there was evidence that it is a favourite abode of otters.

Varanus was very abundant on the bare, rocky south and east coast, nine having been seen when we circumnavigated the island.

No crocodiles were noted.

As regards birds, the striking feature of Ziro was the unusual number of "Open-bill storks" along the rocky shore, and herons also were more noticeable than usual, a species new to me being seen on the E. coast. Small birds were not noticeably absent from Ziro, although the big spiders were present in abundance.

The most striking feature of the Lepidopterous fauna was the great abundance of *Acraeines*, fresh broods of several common species having hatched out just before the time of our visit. Seven of the thirty-two species of butterflies counted were *Acraeines*; nine were *Lycaenids*.

19.—ISENTWA.

Isentwa is formed almost entirely of a grass-clad hill sloping steeply to the water, with shore of rock or shingle. A very small belt of cane on the north shore and a belt of bushes on the west shore form almost all the shelter save for a few fig trees on the rocky face of the south coast.

Hippopotami visit the island, although there can be but little food for them, and have made a track across the top of the hill.

Varanus is common, three having been met with.

None of the large spiders were seen on Isentwa.

Seven species of butterflies were counted, of which five were *Lycaenidae*. The commonest species on the island was a *Lycaenid* which is only occasionally met with on other islands.

20.—LUKALU.

The greater part of Lukalu is a low, grass-clad hill, sloping more steeply down to the water on the west, where the shore is bare and rocky. Along the greater part of the north and east the shore is flat and sandy, and there is a very thick belt of cane. The trees on the island are confined to the south-eastern point where at one time there were plantations. Along the south shore is a belt of E'tungulu and bushes; here the coast is again rocky.

Hippopotami as usual frequent Lukalu.

Varanus abounds, five having been seen, but no crocodiles were noted.

Nothing characteristic was noted about the birds of the island.

In regard to butterflies, *Acraeines* were very abundant in individuals, and five species were noted with seven *Lycaenids*, out of fifteen species of butterflies, which, curiously enough, do not include any species of *Hesperidae*.

Off the south-eastern point of Lukalu are two small flat islets, only separated from each other by a very narrow channel, and from Lukalu itself by about a hundred yards. These islets, which will be known as the accessory Lukalu, do not measure much more than 150 yards in greatest diameter. They have large areas of gravel and coarse sand, and an astonishing variety of herbs. Several kinds of bushes grow thickly in places, and give good shade.

A *Varanus* was seen on one of these islets, also a small crocodile. Weaver birds and sun-birds were plentiful, and the "nightingale flycatcher" was heard.

Three species of *Lycaenid* butterflies were found on these islets.

21.—KIUWA.

A remarkably interesting island, consisting of a conical grass-clad hill rising very steeply out of the water to a height of 318 feet from a base of about a mile. At the north-east end the slope is more gradual, and here are the old plantations now relapsing into jungle, and extending about two-fifths of the distance up the hillside. Along the south-east shore are bold cliffs and rocks; there are two small bays with beach of shingle backed by cliff. There are fairly thick trees and bushes on the eastern half of the south-eastern shore, but the south-western end is very bare; the coast, formed of unbroken rock, rising straight out of the water; the slope to the hill top covered with grass only. The shore that faces north-west has a narrow but thick fringe of bushes growing for the most part on rock; but at the eastern end of this shore there is a good deal of coarse sand covered by bush.

Kiuwa was less frequented by Hippopotami than any island that has been visited; I do not remember having seen any tracks of them.

The grass tussocks covering the hill were very thickly populated

by rodents (as on Kizima, q.v.), and this was quite a feature of the local fauna.

Bats were plentiful, as usual, in the bush.

Crocodiles were fairly abundant on Kiuwa; four were noted when we went round the island. Only one *Varanus* was seen.

Birds along the rocky shore were very few, but a spit of am-batches at the N.E. end provided resting places for many cormorants, etc. In the old plantations sun-birds and weaver birds abounded, but flycatchers were few. A species of song thrush was heard, which, though abundant at Entebbe, had not been met with on any of the islands yet visited.

Twenty-five species of butterflies were noted, and one more on later visits. Lycaenids were the most numerous in species; nine were noted, of which one, quite abundant but local, was not met with on any other island. The five species of Acraeines were extremely abundant in individuals. *Hesperidae* were very scarce, and Satyrines were absent, even from the grassy hill.

A remarkable fact, forming another point of resemblance with Kizima, was that not a single Termite hill was found on Kiuwa.

A single specimen, only, of the huge spiders was seen.

22.—ZIGUNGA.

A pair of rocky islets, grass clad, joined by a narrow isthmus, with very little shade save that provided by fig trees growing on the south-west shore.

A crocodile and two *Varanus* were seen on this tiny islet, and birds (swallows, weavers, and sun-birds) abounded. The "nightingale flycatcher" was heard frequently singing in clumps of a tall composite plant, its favourite habitat.

One Lycaenid and one Acraeine were the only butterflies seen on Zigunga. There were no big spiders.

23.—WEMA.

The large island of Wema was too big to be thoroughly explored during the time at our disposal.

It has three hills, connected by relatively low necks to a central one. This latter has a great deal of forest, but is not universally covered by it. At the north-west point of the island is a flat topped hill with forest on its basal slopes, but its summit grass clad only.

At the south-west point is another flat topped hill, entirely grass clad, at the foot of which our camp was pitched.

A fourth hill, at the eastern end of the island, is conical in shape, and is grassy at the base, only the summit being capped with forest.

The south side of Wema was the only part which we could fully investigate. It slopes steeply down to the water, the lower slopes being covered with the old plantations now becoming jungle and with untouched forest.

A long stretch of shore divided into bays by peninsulæ and small rocky points forms a typical fly beach; that is, a beach (of shingle) backed by dense forest shade.

Beyond the eastern end of the fly beach the shore is formed of flat rock shelving gradually into the water—bare for the most part, but with two localised patches of bush.

Beyond the western end of the open fly beach are two little bays, at the back of which are the old plantations.

Wema, though not so large as Damba or Kome, is able like them to support monkeys.

Hippopotami of course are there, and *Varanus* was common. On an island of such size and with such variety of conditions birds of many species abound. I was particularly struck, however, with the great abundance of the common black and white crow.

Wema was remarkable for the extraordinary abundance of butterflies; on the fly beach and in the jungle behind it were more butterflies than I have seen anywhere. A total of a hundred and twenty-four species were counted. The Lycaenids (23 species), Hesperids (25), and Nymphalines (36) were very abundant in individuals, as were the Pierids, of which 13 species were counted.

The Danaines (4 species) and Acraeines (only 9 species) were extremely poorly represented.

The big spiders were very numerous in the old plantations on the south shore but less numerous in the untouched forest, and in the plantations further away from the water.

24.—MARIDA.

This island consists of two grassy plateaux connected by a flat isthmus some fifty yards wide. This has a ridge of fine pebbles along its eastern shore, fringed with a thicket of dense cane, but its western shore is marshy, with ambatch trees.

This isthmus is well covered with trees and bushes, making good shelter, but elsewhere there is very little shelter for fly along the rocky coast.

There is a good deal of sand in the centre of the isthmus, but not along its coastline.

Hippopotami frequent the islet, and two *Varanus* were seen there. We saw no crocodiles at the time of our visit, but a nest of fifty-eight eggs was found containing young almost ready to hatch.

Birds were plentiful on the island, both bee eaters and fly-catchers were seen in abundance, and many sunbirds.

The big spiders abounded among the thick vegetation of the isthmus.

Butterflies were fairly plentiful. Out of twenty-two species, eight were *Lycaenidae* and eight *Hesperidae*; no Acraeines or Satyrines were seen.

A curious species of slug, with a small fleshy spike at the posterior extremity, was abundant on Marida: it was not noted elsewhere.

25.—SEGE.

A rocky, grass clad islet with very sparse shelter along the shore. But Hippopotami go there, and bats live on it in abundance, and four *Varanus* were seen.

The "nightingale flycatcher" was found on Sege, and swallows and weaver birds were abundant.

No big spiders were noted on this island, and only ten species of butterflies.

26.—WAITWE.

A rocky, glass-clad islet rising to a hill in the centre, but with more shade, provided by fig trees, along its steep, rocky shores than has Sege, and on the west shore there are some thick clumps

of bushes. Here there were also some caves, which provided shelter for many bats. Hippopotami as usual visit this island.

Waitwe resembles Kizima and Kiuwa in that the tussocks of grass on the hill are inhabited by many rodents, whose traces are very conspicuous.

A small python seen in the neighbourhood of the caves probably feeds on these.

Three *Varanus* were seen, but no crocodiles.

As regards birds, bulbuls were more noticeable than on most islands: flycatchers were seen.

Only ten species of butterflies seen, and no big spiders.

27.—WAVUZIWA.

Another small, open islet, with very little shade along the coast line, which is everywhere rocky save for some patches of gravel along the north shore.

The only shade is provided by fig trees growing along the coast and by a few bushes. On the south side of the island there was at one time a small plantation which is now overgrown with weeds and does not afford much shade.

A crocodile and three *Varanus* were seen on Wavuziwa, and the usual tracks of hippopotami.

In spite of the small amount of shade a coucal was found, or rather its characteristic note was heard, and the usual sunbirds and weavers were seen. The "nightingale flycatcher" was abundant.

No big spiders were found on Wavuziwa, and only ten species of butterflies. Large numbers of a Lymantrid larva, of a species not hitherto met with, were quite a feature of the fauna, as also was a species of Coccid, whose large white fluffy masses were very conspicuous on a variety of shrubs and herbs.

28.—YEMPATA.

A large island which we could not thoroughly study owing to lack of time. It has very excellent forest shade all round the shore, except at the north end at some points where there is a good deal of papyrus, and has a relatively small amount of open ground rising slightly in the centre.

A long strip of pebbly beach, for at least half of the length of the west shore, forms a typical fly beach, and there is sandy shore on the south point, but elsewhere the wall of forest is bordered with papyrus, ambatch, and other bushes.

Hippopotami much frequent the old plantations behind the fly beach, and my canoe men said they had seen tracks of the "Enjobe" (Speke's Tragelaph). No monkeys were seen.

Only one crocodile and one *Varanus* were seen on our single visit to the island.

Butterflies were numerous, but it was impossible on an island of large size to make a list from a single visit.

No big spiders were seen, even in the old plantations.

29.—SINDIRO.

A well forested island, with a low grassy hill at the north end, the other end being flat, with a good deal of sand.

The forest forms a dense wall all round the coast, with, in front

of it, thickets of cane, E'tungulu, and ambatch, so that landing is impossible in many places.

There are no fly beaches, there being no foreshore anywhere, and the actual shore line being either rocky or muddy.

At the flat south end of the island are overgrown plantations, with a fairly large open sandy area, which, however, is not visible from the water, owing to the dense belt of cane and E'tungulu surrounding it. This is evidently much frequented by *Varanus*, which had dug many holes in it. No crocodiles were seen. The usual paths made by Hippopotami went through the old plantations.

It was not possible on a single visit to enumerate the butterflies, but *Hesperidae* seemed conspicuous by their numbers in the old plantations, which were visited, since it was the only place where landing could be made for the purpose of fly catching.

The large spiders were very abundant here.

30.—KIBIBI.

An island of the type of Kerenge, but rather more hilly. Most of the forest is on the north side, there being overgrown plantations on the south and east sides.

The coast line is mostly rocky, but there is a bay on the south with light, though hardly sandy, soil; on the north there is some sand, with bushes growing in it.

There are no fly beaches; the forest on the north comes close to the water, with a dense belt of the tall *Salvia* in front.

The west coast has flat shelving rock, with ambatches in the water. The old plantations are much frequented by Hippopotami, of which three were seen in the bay on the south shore. Bats as usual abound.

Two *Varanus* and a crocodile were seen on the south shore, which is mostly bare rock and very suitable for basking on.

Of birds a noteworthy feature was a song thrush, which, though met with on Kiuwa, was not heard on any other island. The red and blue plantain eater, also rarely met with among the smaller islands, was heard on Kibibi.

The big spider does not seem to find conditions on Kibibi favourable, for only a single specimen was seen.

The time at our disposal did not admit an enumeration of all the butterflies of Kibibi, but a species of Nymphaline (*Byblia*) was met with abundantly which was not seen on any other island, though it is common at Jinja on the mainland.

This is a convenient place for noting that in many ways the islands of the Wema group show their affinity with the mainland in the neighbourhood of Jinja, in Usoga; for plants were noticed in abundance on them which are very conspicuous features of the flora of Jinja, but hardly noticeable at Entebbe, or on the islands of the Kome group opposite to Entebbe.

31.—DYAVODEMI.

This small island is mostly covered with overgrown banana plantations, but there is a very little forest growth on the north side. On the south side there is very fine white sandy beach, but elsewhere the shore is either rocky or marshy, and, where rocky, rather bare; where marshy, growing ambatch.

It is frequented by Hippopotami, and a large crocodile was seen

in the same place where one was seen in 1911, when Dyavodemi was visited from Damba. Only one *Varanus* was noted. Bats were, as usual, common in the thick vegetation.

As regards birds, a remarkable feature was the apparent complete absence of weavers, nor were any coucals or flycatchers seen.

A species of lizard, not usually common, was extremely abundant on Dyavodemi; it was a stumpy, short legged, longitudinally striped species, with short tail blue at the base.

Large spiders were extremely abundant in the old plantations, and *Mantidae* were very plentiful also.

Unusually abundant, and a very marked feature of the fauna, were large Asilid flies, and two Phytophagous beetles (*Cassididae*) were in such enormous numbers that in many places the leaves of *Ipomaea* on which they feed had been everywhere eaten except for the veins.

As regards butterflies, Acraeines were in the majority, and six species—all, save one, abundant—noted. Of the total of twenty-five species counted only two were Nymphalines, and *Hesperiidae* and *Lycaenidae* were extremely scarce in individuals.

32.—MASOVWI.

This small neighbour of Dyavodemi, only a few hundred yards away from it, measures about 150×50 yards. It is of grey rock, but has a deposit of sand on the north side, on which grow dense bushes, of the species previously mentioned as Kinsambwe. The western end is bare rock; the eastern end and south shore are low and marshy with open grassy spaces in which grow ambatch bushes, and there is a large thicket of ambatch in the water off the middle of the south coast.

Hippopotami make regular tracks across Masovwi, a large crocodile was seen, and a clutch of fifty-nine eggs discovered in the sand, containing fully formed, but small, young.

No *Varanus* were seen, but small lizards abounded, though the one specially mentioned on Dyavodemi was not seen on Masovwi.

Cormorants and moorhens abounded amongst the ambatch bushes, and weaver birds, though absent from Dyavodemi, were found here, as also were sunbirds. The sweetly singing flycatcher was also seen.

No big spiders were seen, which is interesting, seeing how near is Masovwi to Dyavodemi, where they abound.

Of butterflies, one species of Lycaenid abounded, and one Hesperid and one Pierine were also seen.

33.—MTIOMU.

A very small, rocky islet, in dimensions about 100×20 yards, with a sand spit at one end. Its vegetation is very varied, there being many flowering herbs, and rich growth of creepers over bushes, and at the end opposite to the sand spit, a thicket of ambatch.

Hippopotami visit Mtiomu, and also, probably, crocodiles and *Varanus*, which were seen on other islets close by.

Many cormorants perch on the ambatch, and weavers, sunbirds, and the "nightingale flycatcher" were all noted.

There were no big spiders on the island, and only a single Lycaenid butterfly was noted.

Two conspicuous beetles were very abundant indeed, and gave

the fauna markedly insular characteristics. One was a large black and yellow Cetoniid, the other a black and dull red Longicorn feeding on the green bark of ambatch and Muvuvumye bushes. Both these species are generally distributed, but I have never seen them anywhere in numbers approaching their abundance on Mtiomu.

34.—TOKWI.

Merely a large rock, but not too small to be inhabited by weavers and the "nightingale flycatcher." Quite a number of bushes grow on Tokwi, well covered with *Ipomaea* creeper.

One *Varanus* was seen.

35.—KAWAGA.

This is only a rock about ten yards in diameter, supporting only one kind of shrub—the Muvuvumye. On this were very large numbers of larvæ of a small Chrysomelid beetle, which may usually be found on this shrub, but not in such quantity.

On an adjoining rock, only about twenty yards away, however, there were none of these larvæ, or the beetles, nor were any seen on Mtiomu, which is quite close to Kawaga.

Here again the fauna has markedly insular characteristics.

36.—RUMFUA.

This island lies off the south coast of the west end of Kome, and is therefore rather exposed, there being nothing between it and open lake. It is a rocky island universally covered with thick bush and fern, but has few large trees.

The north-west point is formed by a shingle beach, behind which is a small patch of sand, the only sand found on the island, whose shore is rugged, and formed of bare rock or heaped boulders.

Hippopotami have their regular sleeping places on Rumfua.

It appears to be, probably from the nature of the shore, especially suited for *Varanus*, of which five were seen, but no crocodiles. An otter was also seen.

A large flock of white egrets was noticeable on the north-east shore; but generally speaking, birds were very scarce. No sun-birds or weavers were seen, and only one flycatcher.

This scarcity of birds was again found to be correlated with great abundance of the huge spiders, whose webs stretched in all directions between the bushes, and also of *Mantidae*.

Butterflies were scarce. Two very common Lycaenids were taken, a third species usually found at the edge of forests (*Aphnaeus orcas*), and a forest inhabiting Nymphaline (*Euralia*). The butterfly fauna is therefore rather remarkable.

TABLE 1.

In descending order of size.	Number of species of butterflies.	Infested with spider (in order of size)	Crocodiles seen.	<i>Varanus</i> seen.
<i>Large islands :</i>				
Damba	Wema .124	Wema	Tavu .. 9	Rumfua .. 5
Wema	Dwanga	Sindiro	Ngamba .. 6	Ziro .. 9
Yempata	Mkuru ..65	Kerenge	Kiuwa .. 4	Lukalu .. 5
<i>Medium :</i>				
Bulago	Bulago ..61	Dwanga Mkuru	Kizima .. 2	Sege .. 4
Nsadzi	Dwanga Mto 57	Ziro	Zigunga .. 1	Wavuziwa .. 4
Bulago	Tavu ..47	Dwanga Mto	Masovwi .. 1	Sanga .. 2
Kibibi	Kerenge ..45	Dwasendwe	Sanga .. 1	Isentwa .. 3
Sindiro	Dwasendwe 44	Marida	Wavuziwa .. 1	Kizima .. 2
Kerenge	Kimmi ..40	Dyavodemi	Dyavodemi.. 1	Waitwe .. 3
Dwanga	Sanga ..35	Rumfua	Marida .. 1	Marida .. 2
Mkuru	Ziro ..32	Kisigalla	Kerenge .. 1	Kerenge .. ?
<i>Small :</i>				
Dyavodemi..25	Sanga		Yempata .. 1	Ngamba .. 3
Kiuwa	Kiuwa ..25			Dwanga Mto 2
Ngamba	Kisigalla ..22			Enkusa .. 1
Kimmi	Marida ..22			Bulago .. ?
Ziro	Ngamba .. 22			Tokwi .. 1
Dwanga Mto	Lula ..15			Lula .. 1
Dwasendwe	Lukalu ..15			Dyavodemi 1
Waitwe	Waitwe ..10			Tavu .. 1
Tavu	Wavuziwa ..10			Dwasendwe 1
Marida	Sege ..10			Kimmi .. 1
Dyavodemi	Isentwa .. 7			Kiuwa .. 1
Rumfua	Rumfua .. 6			Dwanga
Wavuziwa	Kizima .. 4			Mkuru .. 1
Isentwa	Masovwi .. 3			Sindiro .. 1
Kizima	Zigunga .. 2			Yempata .. 1
Sege	Nkusa .. 1			
Lukalu	Mtiomu .. 1			
<i>Very small :</i>				
Kisigalla				
Sanga				
Lula				
Masovwi				
Zigunga				
Maungwe				
Mtiomu				
Nkusa				
Resuvu				
Tokwi				
Kawaga				

In these two columns the islands have been put in descending series according to the number of crocodiles or *Varanus* found on them in proportion to their size. Comparison of these two columns with the column showing the percentage of wing ♀% in Table II. is of much interest.

THE DEGREE OF INFESTATION OF ISLANDS BY *GLOSSINA*.

An account will now be given of the degree to which each of the thirty-six islands visited was infested by *Glossina palpalis*. In order to compare one locality with another it seems best to take as an index the number of *males* caught per boy per hour, as was suggested by Mr. Fiske, rather than the total number of flies caught, owing to the great variations of the percentage of females caught. Mr. Fiske has shown that the males are always

at first caught in greater numbers, being apparently more active and readily attracted by "curiosity," and can be caught from off bushes on which they bask; whereas only those females which are actually *eager to feed* are caught by the boys.

Thus, on Tavu, when going along a track just made by canoe-men through thick Kinsambwe bushes, whose leaves had often been turned over to show their silvery under surface, I frequently noted male flies settled on these conspicuous places. I also tried the experiment of holding my (white) net so that I could catch the flies that settled on its rim. In one hour I thus caught twenty-six males and only six females. I then caught from off myself those flies which, by lowering their proboscis, showed their desire to feed, and caught ten females to eight males.

It is therefore necessary to withdraw my previous statement in my Second Report (Reports of the Sleeping Sickness Commission, No. xiv., 1913, p. 9, at foot) "that it is only the attractiveness of a fly boy as a source of food that leads to a fly being caught." This statement therefore refers only to females and *hungry* males; some fly boys who catch flies more from off bushes than from off their clothes, catch more males than the, usually more expert, boys who are adept at catching flies from off themselves.

In any area where, by catching large numbers of flies consecutively, their numbers could be reduced, it was always found that as the number of males caught per boy per hour diminished, the female percentage rose, without any rise being noted in the number of females caught per boy per hour. That is, the increased female percentage is entirely due to the more curious and active males being caught and so diminished in numbers. Thus, on Tavu, flies were continuously caught in one locality on two successive days.

Date.	Time.	Total catch.	♂ ♂ per boy hour.	♀ ♀ per boy hour.	♀ %
Jan. 15th, 1914..	7.45—8.45	473	62.5	7.5	10.7
" " "	9—10	303	36.7	4.3	10.5
" " "	10.15—11.15	235	23.5	6.5	21.7
" " "	11.30—12.30	216	20.3	6.8	25.
" " "	1.30—2.30	222	20.3	7.5	27.
Jan. 16th, 1914..	7.45—8.45	275	33.	5.7	14.7
" " "	1.25—2.25	241	21.	11.4	35.2
" " "	2.45—3.45	128	9.8	7.1	42.

It is seen that by the beginning of the second day the proportions had not returned to their original values, and by the end of the second day, when the numbers of the males had been greatly reduced, but the females were as numerous as at the beginning of the first day, their percentage in the total catch had gone up to 42%!

For the record of the infestation from any island that was visited frequently the *maximum* catch was taken as the index. For by frequent catches, as has been shown, the number of flies may be reduced and the percentage of females raised, so that an average of many catches would not give a fair record to be compared with other places where only a single catch was made.

BULAGO.—(January 17th—22nd).

Locality.	No. caught.	♂ ♂ per boy hour.	♀ ♀ per boy hour.	♀ %
N. bay	271	43.4	10.8	19.9
Sandy S. point	422	36.6	28.2	45.
Sandy S.W. shore	223	20.6	14.	40.4
Forest on N. point	103	6.8	4.7	40.8
Average		26.8	14.5	36.5

The low percentage of females at the N. bay locality, where, judging by the males, the flies were most numerous, is remarkable. The small numbers of the fly in the forest at that time are also noteworthy.

Breeding grounds are very extensive on Bulago. The sandy southern point everywhere offers abundant facilities, as also the sandy south-west shore, and the shore of the north bay. This latter is of interest because from the water the coast appears marshy and quite unsuitable, but on the landward side of the belt of bush the soil is sandy and eminently suitable.

Later on there was discovered on the western shore of the forested north point an open sandy area among rocks where pupæ were to be found in some abundance. Also on the north shore at its western end is a piece of well shaded sandy foreshore eminently suitable for a breeding ground, and pupæ were discovered here in numbers in 1918. In the strip of jungle along the shore of the north bay I several times found a few pupæ collected at the base of large, hollowed out, tree trunks, where the light, loose soil was kept dry. These sites were often indicated by the conical pits of "ant lions."

Locality.	No. of Pupæ found per boy per hour.
N. bay	8.5
S. point	7.1
S.W. shore	5.4

KIZIMA.

Visited January 14th. Unfortunately very careful figures were not kept, but a total of 99 flies was caught on the two sides of the island, giving ♂♂ per boy per hour (for brevity's sake this will be subsequently alluded to as ♂ b.h.) as 2.6 and ♀ % as 11.

Only 6 pupæ could be found in about 10 boy hours! This is of interest because there are good potential breeding grounds on both east and west sides of the island in well shaded sand. The small numbers of fly on Kizima cannot be due to lack of facility for breeding, and must be due to some other factor. I am inclined to think the amount of shade is insufficient.

TAVU.—(January 15th—17th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
Open space amongst bushes on W. coast	473	62.5	7.5	10.7
Open space amongst bushes on E. coast	339	33.8	10.7	24.
Average.. ..		48.1	9.1	17.3

The main breeding ground on Tavu is on the west shore, where, at about the centre of the length of the island, is a ridge of coarse brown sand which was thrown up in former days when the lake was at a higher level. It is quite invisible from the water, being now separated by rocks from the water's edge. It is very well shaded by a dense growth of Kinsambwe bushes, and, where best developed, is not 100 yards in length (see figs: p. 67). On the eastern shore there is also the sand left by the receding lake, but it is not nearly so suitable for a breeding ground, being firmer, moister, and less well shaded, and to a much greater extent intermingled with other soil, than on the W. coast.

Locality.	Pupæ per boy hour.
W. side of Isle	74
E. " "	18

The number of pupæ found per boy per hour on the western breeding ground was greater than had ever been found anywhere else. This is apparently due to the favoured breeding ground being of small area, so that the pupæ were thickly concentrated. At one spot one boy collected, without moving, 268 pupæ in an hour and another boy, close by, found 138. This does not include the empty puparia, but only living pupæ. These, and the sandy south point, are the only places on the island (which has been fairly thoroughly explored since the first visit) which can be called breeding grounds, but here and there among beds of E'tungulu the soil appeared light enough, though doubtfully always dry enough, to furnish nooks where scattered pupæ might be found by laborious search.

RESUVU.

Has no *Glossina* on it. When I first visited the island (January 17th) the reason was not apparent, for nothing then seemed lacking as regards food and shelter, and the whole of the island was formed of sand and gravel which seemed to provide excellent breeding grounds. At that time, however, the level of the lake was low, and it has since risen considerably so that on rough days the island is nearly covered, hence it is probably because pupæ could not live on the island that *Glossina* is unable to find a footing. At a later visit in 1918 the islet was found completely submerged.

NSADZI.—(February 3rd—4th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
S. fly beach	391	36.4	7.	16.1
N. shore at E. end	51	23.4	2.	7.9
N. shore at W. end	262	20.	9.1	31.3
E. forest	249	12.	2.9	19.5
N. forest	127	11.7	7.4	38.7
Average.. ..		20.7	5.7	28.4

The great variation in the percentage of females caught at different localities on Nsadzi is very noteworthy.

As regards breeding grounds on Nsadzi, the sandy shore of the S. beach, well shaded by forest, has the characteristics of a breeding ground of first quality. Pupæ per boy per hour 14.9 here.

At the western end of the north shore there is a good deal of sandy soil, but the shade is perhaps not adequately distributed, for very few pupæ could be found there, only at rate of 1.7 per boy per hour. The forested eastern end of the island has no breeding ground. The northern shore is entirely rocky, the southern either rocky or marshy. The vegetation on this south side is so extremely dense that there is a thick wall of matted cane and creeper and tall grass through which one cannot penetrate, and along the margin of the water grow ambatches. Attempts to discover any breeding ground by coming to the coast from the interior, have failed.

I believe that the degree to which the isolated eastern forest is infested with flies represents the numbers to which the fly can attain in the absence of breeding grounds, when it has to deposit its larvæ in various nooks and crannies among rocks, tree roots, etc. To this point I shall return later.

KIMMI.—(January 24th—27th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
S.E. grazing ground	366	38.	43.	53.3
N. grazing ground	332	37.1	36.6	49.7
E. shore	635	33.	37.3	53.2
Forest	410	28.9	17.2	37.3
S.W. shore	354	22.2	56.2	71.8
Average.. ..		31.8	38.1	53.1

The remarkably high percentage of females caught on Kimmi is very noteworthy. Although the total number of flies caught per boy per hour is greater than on Tavu, yet, judging from the males alone the fly is not so abundant, the difference being made by the extraordinary number of females that were caught. On the hypothesis stated previously the flies must have had little facility for obtaining food at the time of our visit. Comparison with Tavu is interesting.

We were certainly never attacked anywhere by the flies to such a degree as on Kimmi; they came off in a swarm to the canoe before we touched the shore; and during our whole visit we were continually bitten. As will have been seen from the general account of this island and Tavu, the latter seemed to have a much better supply of reptilian blood for the fly than did Kimmi.

As regards breeding grounds, a very large part of the coast of Kimmi has shaded, sandy areas either close to the water or a few yards back. In addition the large caves, with dry, sandy, or gravelly floor, at the south-west point, and rocks that have been undercut at their bases, provide admirable breeding grounds. Probably on account of this great extent of breeding ground pupæ could nowhere be found in large numbers; here again is an interesting contrast with Tavu island.

Locality.	Pupæ found per boy per hour.
E. shore	7.3
N.E. shore	7.
In caves	6.7
Just outside caves.	2.9

It may be noted that in the caves very large numbers of *empty* puparia could be found, but only living pupæ are counted above.

NGAMBA.—(January 30th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
E. grazing ground	141	15.4	0.2	1.3
N. shore	165	12.9	1.9	12.8
S. shore	52	10.	0.6	3.8
S.E. point	44	9.5	0.0	0.0
W. shore	55	5.8	0.3	4.7
N.W. point	50	4.5	1.	18.1
S.W. point	20	4.	0.4	9.
Average		8.9	0.6	7.1

Flies were caught at a series of localities all round Ngamba, which, except at the rather exposed S.W. and S.E. points, seemed to offer everywhere good shelter. The maximum number was taken on the E. grazing ground, a stretch of marshy shore with lush grass, and clumps of bushes behind which was a belt of E'tungulu bordering the forest. It is interesting to note that the bushes had been eaten by the Sitatunga antelope.

As regards breeding grounds, they were limited to a small part of the northern shore and the north-west point. Although in proportion to the size of the island there was as much breeding ground as on Tavu, yet it was not of such good quality, not being nearly so well shaded (see figs: 1 and 2, p. 66).

Locality.				Pupæ per boy per hour.
N.W. point	..	..	..	2.5
N. shore	..	..	..	1.7

I am inclined to think that were the breeding grounds of Ngamba more extensive and of better quality the fly, which has everything it wants in shelter and food supply, would be very much more abundant on this island.

SANGA.—(February 5th.)

Glossina was present only in minimal quantity on Sanga, at the rate of 0.2 males per boy per hour. This was, at first, surprising, seeing that the island is covered with forest, but search showed that there were no places that could be called breeding grounds, though there was one patch of light soil. As has been said it was a "Spider island," but the question of the importance of the spider will be discussed later.

LULA.—(February 11th.)

Glossina is remarkably abundant on Lula, if it is borne in mind that nowhere is there any sand or light soil that could be called a breeding ground on this rocky little islet.

No. caught.	♂ b.h.	♀ b.h.	♀ %
205	6.8	2.3	25.3

The comparatively high percentage of females caught is perhaps explicable, as Mr. Fiske pointed out to me, by the fact that in November, 1913, he visited the island and his fly-boys caught flies continuously so as to reduce the ♂ b.h. figure from 13.7 on November 18th to 0.6 on the 27th, the ♀ % rising simultaneously from 6 to 75.7!

This effect of continuous catches shows well Mr. Fiske's point that the disparity of sex is *apparent* only, the females being there but not coming to be caught so readily as the males. Possibly at the time of our February visit the sexes had been not quite restored to their normal proportions.

The flies on Lula deposit their larvæ in light vegetable humus sheltered by overhanging rocks, for there are no other suitable localities. Lula is thus particularly interesting for it shows that fly can live on it in some numbers without a definite breeding ground.

KISIGALLA.—(February 11th.)

There are extremely few flies on this island.

No. caught.	♂ b.h.	♀ b.h.	♀ %
71	1.7	0.6	26

There is excellent shade all over Kisigalla, and there is no reason from what we saw to doubt sufficiency of food. Quite a good potential breeding ground was found in a bed of E'tungulu growing in sandy soil. It seems likely, therefore, that the abundance of big spiders may be the reason for the scarcity of fly.

DWANGA MKURU.—(February 6th.)

Glossina is, for all practical purposes, absent. Mr. Fiske and I and 9 fly-boys, during a visit from 9 a.m.—4 p.m., only saw a single fly, which may possibly have journeyed there with us. This may be connected with the fact that nowhere was there a breeding ground; also it was a spider island.

DWANGA MTO.—(February 9th.)

The same may be said of this as of its larger neighbour. The 9 fly-boys caught 4 males and a single female on the day we were there.

DWASENDWE.—(February 10th.)

Not a single *Glossina* was discovered, but, though generally like its neighbours, this island has a small but good potential breeding ground; an area of dry, well sheltered sand. But there was not even an empty puparium to be found here. Like its two neighbours Dwasendwe is a spider island.

MAUNGWE AND ENKUSA.—(February 13th.)

No *Glossina* were found on either islet, they are probably too small.

DAMBA.—(February 12th.)

It was only possible to investigate the infestation of Damba along the S.W. half of its southern shore; from the flybeach just south of the equator to the south-west point. The fly-beach is called locality 1; loc. 2 is about half-a-mile away on the shore of the bay to the west of the fly-beach, and loc. 3 at the head of that bay; loc. 4 at the S.W. point of the island, loc. 5 on the same end of the island but facing the channel between Damba and Kome, and loc. 6 behind the belt of papyrus which lines that channel.

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
1	269	55.8	4.	7.1
6	138	27.3	3.3	10.8
2	132	25.5	3.8	13.
4	254	24.3	3.9	10.3
5	63	23.5	4.4	10.8
3	119	22.7	3.8	14.3
Average ..		29.8	3.9	12.

The remarkable thing about the above table is the great constancy of the ♀ b.h. figures. This, being constantly low, seems to indicate a plentiful supply of food all along the coast examined. In my second report (see No. 14 of Reports of the Sleeping Sickness Commission, p. 20) I showed that of 177 flies which were found to contain fresh blood on Damba 84.8% had fed on non-mammalian blood. This is the more interesting in view of the facts to be brought forward later, showing that the flies much prefer reptilian to mammalian blood. It appears, however, that in other localities they are unable to obtain reptilian blood and a greater percentage of mammalian blood is found (loc. cit.; figures for Bugalla and Jinja). On Damba the *Sitatunga* was plentiful enough, but the flies seemed to prefer reptilian blood.

The whole of the coast which was examined supplied at intervals breeding grounds of high quality, as well as the one which supplied several thousands of pupæ monthly for the Mpumu laboratory. One such was at locality three, where a clump of date palms grew on a sand bank. This large extent of breeding grounds explains why the continued drain made by Mpumu had no effect on the fly.

Locality.	Pupæ per boy per hour.
Fly beach (1)	39.3
3	8.7

KERENGE.—(February 15th—17th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
In E'tungulu belt N. shore..	155	9.	2.5	21.7
Point on E. shore, formerly a landing place	57	5.1	2.	28.2
N. point	21	3.8	0.4	9.5
Old plantations	38	2.8	1.	26.3
E. bay	12	1.6	0.8	33.3
W. bay	11	1.	1.2	54.5
Average		3.9	1.3	28.9

As regards breeding grounds, the most productive place was at the eastern landing place where was an accumulation of sand amongst, and on the top of, flat rocks. The shade was chiefly produced by masses of the aromatic labiate which flourishes everywhere under such conditions. At the head of the E. bay was a large area of light soil, where the E'tungulu plant grew thickly and made good shade. It was surprising that more pupæ were not found here.

Locality.	Pupæ per boy per hour.
E. landing place	4.7
W. bay	1.1
E. bay	0.7

ZIRO.—(February 14th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
S. E. point	148	9.7	1.3	11.5
Old plantations, N.E. point.	162	8.3	3.7	30.9
Old plantations, S.W. bay .	23	2.2	0.3	12.
N. shore	7	1.3	0.2	13.3
Average		5.4	1.4	17.

Pupæ were not collected on Ziro, but Mr. Fiske, who walked round the coast while I examined it by canoe from the water, reported that along the N. shore in the thickets of cane and E'tungulu there were places that seemed suited for pupæ.

ISENTWA.—(February 16th.)

No *Glossina* were found on Isentwa; their absence is almost certainly due to deficiency of shade.

LUKALU.—(February 16th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
N. shore, E. end	107	16.4	5.	23.4
S. shore, E. end	26	8.8	1.6	15.4
S. shore, W. end	11	3.2	1.2	27.
Average		9.4	2.6	21.9

The flies on Lukalu were of course confined to those parts of the shore that were shaded, and were most plentiful in the vicinity of the east point where the trees and bushes were biggest. The whole of the cane thicket along the N.E. shore is such an admirable breeding ground that one could not expect to find any collections of pupæ. Hence the figure per boy per hour is only 1.7.

KIUWA.—(February 19th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
E. point, N. side	245	22.2	5.	18.4
E. point, S. side	85	16.	2.9	15.3
S.E. bay (beach)	58	12.2	0.7	5.4
Old plantations	125	9.9	4.	29.
Average		15.1	3.1	17.

Considering that shelter for the fly is almost confined to the region of the plantations at the east end of the island, with the exception of a very thin fringe along the east half of the north shore and the strip at the back of the beach, *Glossina* may be said to be quite unexpectedly numerous on Kiuwa. This is the more surprising when the difficulty of finding pupæ is taken into account.

Two localities were found that seemed at first sight to be excellent breeding grounds; one, a strip of coarse sand in which grew thick Kinsambwe bushes on the north side of the eastern point; the other in the bay on the south coast where waves had previously undercut the foot of the cliff, the resulting small caves now providing well sheltered dry sand.

Yet in the former locality only 20 pupæ could be found, representing 1·3 per boy hour, and in the latter only 15, representing 3·3 per boy hour. Prolonged searching revealed no other breeding ground on the island, but here and there collections of empty puparia could be found among rocks, and in one such locality I found three living pupæ and four empty puparia.

A possible reason why the first place mentioned was not really a good breeding ground is that the surface was very thickly covered with fallen leaves, and the shade being very dense these had never dried, and smelt mouldy. I have previously laid it down as a *sine qua non* for a good breeding ground that the surface must be *well ventilated*, and quite recently I have obtained further evidence on this point. On Tavu island, beyond the end of the ridge of sand which was described as the main breeding ground, there was more sand lying at a lower level and very densely covered with vegetation so that its surface was covered by a mass of damp débris. Here no pupæ had been found, but by the process of searching the surface of a small area (about eighteen inches square) had been cleared of débris, and the sand left ventilated, though the shade was not interfered with appreciably. *A fortnight later I found about two dozen freshly deposited pupae in this very spot!*

ZIGUNGA.—(February 20th.)

There were no flies on Zigunga—possibly it was too small. The shade which was provided in places by fig trees along the coast was quite good enough to have sheltered *Glossina* had it been on a larger island where there was more and better shade as well. There was a good food supply, but no good breeding grounds.

WEMA.—(February 23rd—25th.)

The only part of Wema that was worked for fly was the strip of the south coast where there was continuous forest and jungle down the slope of the hill to the shore. Six localities were chosen here, numbered from W. to E.:—

- 1.—A patch of elephant-grass growing in sand behind ambatches and backed by bush.
- 2.—A beach of red pebbles in a small bay behind ambatches and backed by an old plantation.
- 3.—A similar locality, but without ambatch.
- 4, 5, 6.—The “fly-beach” formed of an open foreshore of red pebbles backed by forest and overgrown plantations growing up the very steep hillside, which in places formed cliffs abutting on the beach. This strip was roughly divided into three portions by small jutting out points of rock.

Beyond the eastern end of the fly-beach were two isolated patches of bush on the shore, separated on the landward side by open grassland, and along the coast by bare, rocky shore. These are numbered respectively 7 and 8, the latter being smaller.

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
2	203	67.	34.5	34.
3	174	59.5	27.5	31.6
4	147	56.5	23.	28.9
6	137	35.6	19.2	35.
5	182	35.2	37.6	51.6
1	61	14.	16.5	54.1
7	78	9.5	10.	51.2
8	40	7.	3.	30.
Average ..		35.5	21.4	39.6

Glossina was most numerous at loc. 2, where, judging by the males, they were more numerous than at any other place that has been met with. But from the second to the sixth locality they were everywhere extremely abundant, although when one penetrated a few yards back into the jungle there was at once an extraordinary falling off in numbers. This serves to illustrate once again the well-known fact that a stretch of open foreshore backed by forest is the most favoured locality for the fly (cp. fly-beaches on Bugalla, Damba, Yempata and Nsadzi islands). The falling off of numbers in the two isolated localities 7 and 8 is interesting. The percentage of females is seen to be very high at some localities, and we found the flies very hungry and persistent in their attacks. Breeding grounds were extensive—practically the whole of the coastline examined was good breeding ground from locality 2—6 inclusive. At loc. 1 the sand was not extensive, and rather moist, so that not many pupæ were found.

The beaches of red pebbles at localities 2 and 3 were good, but most of the pupæ at locality 3 were obtained at the base of a large rock, with sides about 18 and 9 feet long, which was roughly quadrangular and lay with one angle pointing to the water. The two sides facing the water had been cut away at the base when the lake was formerly at a higher level. On the summit of the rock (which was derived from the cliff at the back) grew a fig tree, throwing ample shade.

At localities 4, 5, 6 pupæ were found under the shade of thick vegetation growing among fine red pebbles. There was one remarkably interesting spot; a leafy vine had dropped a long spray from a tree overhanging the beach, which came right down to the pebbles so that its foliage sheltered a small area, and at this spot a number of pupæ were found.

Locality.	Pupæ per boy per hour.	Empty puparia per boy per hour.
3	72.4	146.
4	72.	70.7
5	64.6	47.4
6	49.4	115.8
2	12.4	20.4
1	8.4	20.4

It is seen that as many pupæ were found per boy per hour at 3 and 4 as on Tavu, but the localities were very much more

extensive, so that the breeding grounds on Wema must provide for enormous numbers of flies and probably are responsible for the great abundance of *Glossina* on the fly-beach.

MARIDA.—(February 21st.)

Flies on Marida were practically confined to the well shaded isthmus.

No. caught.	♂ b.h.	♀ b.h.	♀ %
37	4.1	0.5	10.8

The small number of fly cannot be due to lack of breeding grounds which are, potentially of magnificent quality, and extensive; the cane belt on the eastern shore of the isthmus giving apparently all that is needed. Mr. Fiske found some pupæ under shelter of waterworn rocks on the west side of the isthmus. Marida being a spider-island will be further discussed later.

SEGE.—(February 26th.)

Only twenty-seven flies, all males, were caught on Sege by nine boys in an hour. The only spot that could be found that was suitable for pupæ was at the foot of one large, undercut rock on the east side, where there was fine, dry, well sheltered soil. Here one pupæ and a few empty puparia were found.

WAITWE.—(February 26th.)

The only *Glossina* seen were among the bushes in front of the caves on the west, where 6 males were caught by 9 boys in half-an-hour. The only possible breeding ground was on the floor of the caves, where among pebbles and fine soil a few empty shells were found.

WAVUZIWA.—(February 21st.)

One hour's catch by the 9 boys produced six males and one female. No pupæ could be found anywhere, nor was there any place that could be called a breeding ground.

YEMPATA.—(February 24th.)

It was impossible to make an adequate fly survey of this large island in the time at our disposal, and attention was confined to the S. and W. beaches and to the old plantations behind the latter.

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
S. beach	377	59.6	31.6	34.6
W. beach	614	43.5	24.8	36.3
Plantations	207	26.8	19.1	41.6
Average		43.3	25.2	37.5

Flies were thus found to be very numerous on Yempata, and they were very persistent and hungry. It will be noted that the female percentage is high.

The abundance of *Glossina* on Yempata is, I think, amply explained by the large areas of first quality breeding ground provided by the sandy beach on the S. shore, and the pebbles mixed with coarse sand on the W. shore.

Locality.	Pupæ per boy per hour.
W. beach	56.
S. beach	26.7

Seeing that there were such long stretches of good breeding ground and that therefore one would not expect any very great concentration of pupæ, these figures imply that they are present in very large numbers.

SINDIRO.—(February 28th.)

A brief visit on one day was not enough to enable a full investigation of the fly to be made on this well forested island. But the degree of infestation was very much less than had been expected.

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
Plantations on S. point ..	41	15.2	1.2	7.4
W. coast	18	6.4	0.8	12.5
Average		10.8	1.	9.9

Sindiro was a spider-island, which *may* account for its scarcity of fly. Pupæ could only be found on the S. point, where there was a large, well shaded, sandy area, at the rate of 1.8 per boy per hour.

KIBIBI.—(March 2nd.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
N. shore	89	18.6	11.	37.1
S. bay	127	14.2	2.4	14.4
E. shore	33	9.6	3.6	27.3
N.E. point	26	2.2	3.	57.7
Average		10.1	5.	34.1

Breeding grounds on the whole are not good on Kibibi, which may be a reason for the scarcity of fly. The S. bay has fine soil but it holds moisture too much. Here and there, however, where it is lightened by pebbles one could find a few pupæ. There were no breeding grounds on the E. shore. On the forested N. point,

however, there was fair breeding ground, formed by a bank of sand well shaded by Kinsambwe bushes. Here pupæ were found at rate of 9.2 per boy per hour. The W. shore was flat and rocky and unsuited for pupæ.

DYAVODEMI.—(March 4th and 6th.)

Locality.	No. caught.	♂ b.h.	♀ b.h.	♀ %
Bush on N. shore	30	13.	2.	13.3
S. bay	19	6.	0.3	4.8
S. shore	16	4.8	1.6	25.
N.E. marshy shore ..	22	4.8	4.	45.4
Average		7.1	1.9	22.1

Since Dyavodemi had breeding grounds which ought to have provided more flies than were actually found, the scarcity of *Glossina* may perhaps be due to its great infestation by spider.

As regards breeding ground, on the S. shore there was very fine white sand, well shaded by thick bushes. But prolonged search by Mr. Fiske, myself, and 9 boys for three-quarters of an hour only produced six empty puparia!

On the E. shore a thick patch of Kinsambwe bushes growing in much coarser brown sand (the detritus of ironstone rock) provided an apparently ideal breeding ground, but only a few shells and no pupæ could be found here.

In the bush on the N. point were sandy patches here and there. Empty puparia were found here in some quantity (39.2 b.h.), but only one pupa, which was the only one found on the island.

MASOVWI.—(March 6th.)

On this small neighbour of Dyavodemi, five boys in three-quarters of an hour caught 8 ♂ (and no female) flies, = 2.1 b.h. This, apparently, nearly represented the total number of flies on the island, for a second catch of half-an-hour only resulted in one more male! One pupa and four empty shells were found.

MTIOMU.—(March 6th.)

Fly being present on Masovwi I was a little surprised not to find any on Mtiomu. It is, however, smaller, and the shade less adequate.

TOKWI AND KAWAGA.—(March 6th.)

These being merely large rocks cannot be expected to have fly on them.

RUMFUA.—(March 21st.)

Prolonged search by nine boys failed to discover any *Glossina* on Rumfua. There was an entire absence of anything of the nature of a breeding ground, save for a few square feet of sand, not well shaded, at the base of the shingle point. Rumfua is, as has been said, very markedly infested with big spiders.

TABLE II.

♂ ♂ per boy hour.	♀ percentage.	Amount of shade.	Extent of breeding ground.
Tavu 48.1	Kimmi . . .53.1	<i>Class I. (generally well covered) :</i>	<i>Class I. (20-40 per cent. of coast) :</i>
Yempata ..42.6	Wema .. .39.6	Damba	Yempata
Wema .. .35.5	Yempata ..37.5	Dwasendwe	Bulago
Kimmi .. .31.8	Bulago . . .36.5	Rumfua	Kimmi
Damba . . .29.8	Kibibi .. .34.1	Kisigalla	Ziro
Bulago . . .26.8	Kerenge . .28.9	Sanga	Kizima
Nsadzi . . .20.7	Nsadzi . . .28.4	Lula	Lukalu
Kiuwa . . .15.1	Kisigalla ..26.	<i>Class II. (relatively small open spaces here and there) :</i>	Masovwi
Average ..13.1	Lula .. .25.3	Ngamba	Mtiomu
	Average ..24.	Kimmi	Nkusa
Sindiro . . .10.8	Dyavodemi ..22.1	Dwanga Mto	Resuvu
Kibibi .. .10.1	Lukalu . . .21.9	Tavu	<i>Class II. (10-20 per cent. of coast) :</i>
Lukalu . . .9.4	Tavu .. .17.3	Masovwi	Sindiro
Ngamba . . .8.9	Ziro .. .17.	Mtiomu	Nsadzi
Dyavodemi ..7.1	Kiuwa . . .17.	Nkusa	Kiuwa
Lula .. .6.8	Damba . . .12.	Resuvu	Marida
Ziro .. .5.4	Kizima . . .11.	Tokwi	Dyavodemi
Marida . . .4.1	Marida . . .10.8	Kawaga	Kisigalla
Kerenge . . .3.9	Sindiro . . .9.9	<i>Class III. (large open spaces but good shade on shore) :</i>	<i>Class III. (5-10 per cent. of coast) :</i>
Sege .. .3	Ngamba .. .7.1	Wema	Kibibi
Kizima . . .2.6		Yempata	Kerenge
Masovwi . . .2.1	Compare this column with the columns in Table I. showing prevalence of reptilian food supply.	Bulago	Ngamba
Kisigalla . . .1.7		Kibibi	Tavu
Waitwe . . .1.3		Sindiro	<i>Class IV. (1-5 per cent. of coast) :</i>
Wavuziwa . . .0.7		Kerenge	Dwasendwe
Sanga .. .0.2		Dwanga Mkuru	Waitwe
Dwanga Mto ..0.7		Ziro	Sanga
Dwasendwe ..0.		Dyavodemi	Zigunga
Rumfua .. .0.		<i>Class IV. (mostly open but shade for fly at intervals) :</i>	<i>Class V. (no breeding ground) :</i>
Isentwa .. .0.		Nsadzi	Dwanga Mkuru
Zigunga. . .0.		Kiuwa	Dwanga Mto
Maungwe . . .0.		Waitwe	Rumfua
Mtiomu . . .0.		Marida	Wavuziwa
Nkusa .. .0.		Wavuziwa	Lula
Resuvu . . .0.		Kizima	Isentwa
Tokwi .. .0.		Sege	Sege
Kawaga .. .0.		Lukalu	Maungwe
		Maungwe	Tokwi
		Zigunga	Kawaga
		<i>Class V. (almost without shade for fly) :</i>	N.B.—Damba and Wema not included in table as they were only partly examined.
		Isentwa	

FACTORS INFLUENCING THE DEGREE OF INFESTATION.

Taking as an index the figure as calculated for each island the average degree of infestation is represented by 13.1 ♂ b.h.

Out of twenty-five islands on which was found *Glossina* (Dwanga Mkuru, on which one only was found, is not counted),

seven were considerably above the average, and one, Kiuwa, just above. The remaining seventeen range downwards very gradually from 10.8 to 0.07.

The factors that conceivably may influence the degree of infestation may be listed as follows (see Tables I. and II.):—

I. *Topography of Island.*

- A. Size.
- B. Conformation.
- C. Character and amount of shade.
- D. Character of coast.
- E. Amount and quality of breeding grounds.

II. *Inter-relation with other animals.*

III. *Food supply.*

IV. *Climate.*

TOPOGRAPHY.—A. SIZE.

Size alone does not appear to be a factor of any great importance. There certainly, however, does seem to be a minimum necessary. Thus on the tiny islet of Mtiomu, which appeared to be in every way suitable as regards shelter, breeding grounds, and food supply, *Glossina* was absent.

Islands of the size of Lula and Masovwi were the smallest on which fly was found, and Dwanga Mkuru was the largest found not to be infested.

It does not follow that the largest islands must be most heavily infested, for Kome (not included in the table because it has not been sufficiently studied) would certainly be very near, if not below, the average.

It is impossible to say how much importance is to be attached to the size factor owing to other disturbing factors. For instance, Kimmi and Ngamba are of approximately the same size and both well covered with shade. Kimmi has, however, nearly four times as many flies as Ngamba, which is probably due to superiority of breeding grounds.

TOPOGRAPHY.—B. CONFORMATION.

No particularly favourable conformation of an island can be definitely pointed out.

The eight islands that had above the average number of *Glossina* were of a variety of types.

One type is the flat island covered with jungle or forest, such as Tavu, Kimmi, and Damba: the opposite type is that of Wema, Bulago, and Kiuwa, in which there is hilly ground in the centre, often completely bare of trees or bush, but with adequate shade along the shore. It may be said, however, that even those hills that are forested to a greater or less degree have very few fly on them, although on Wema there were some fly throughout the forest which covered the hill side from the water up to about 200 feet elevation.

It may, I think, be said that some stretches of sand or gravel near the water, to form breeding grounds, are necessary if the fly is to be above the average number—although it must, in this

connection, be remembered that these apparent breeding grounds did not seem to commend themselves to the fly on Kiuwa, which was infested to a degree just above the average.

A shore that is very densely covered with bush or cane is not generally so productive as an open fore-shore backed by good shade. Papyrus is generally an indication of the former type of shore.

One type of island can be said to be definitely unfavourable for fly; that illustrated by Rumfua, Dwanga Mkuru and Mto, or Sanga.

These are islands of rock, broken into boulders of irregular size along the coastline, and well covered with trees and bush.

A feature of such rocky islands is the luxuriant growth of large clumps of ferns, both along the shore and elsewhere; indeed, when I saw such a shore I never expected to find many *Glossina*.

It is possible that such islands are unfavourable because they do not provide adequate breeding grounds, and yet Lula, which is rocky but not ferny to a marked degree, has flies which can carry on their race by depositing larvæ amongst the rocks.

The presence of the huge spiders in great numbers on the islands mentioned above (except Lula) introduces a disturbing factor into this, as into many other, questions!

• TOPOGRAPHY.—C. SHADE.

It is a truism that *Glossina palpalis* requires shade, but exactly how much is a difficult question to answer.

I think there is every probability that, on certain islands, the conditions being apparently otherwise favourable, a deficiency of shade accounts for absence or small numbers of fly.

It seems as if a certain *minimum amount of good shade in proportion to the size of the island* was necessary to the fly.

Kizima is a case in point; almost the only example that can be given, for on other islands where fly was scarce the reason may be deficiency of breeding ground, as on Isentwa and Waitwe. But on Kizima both breeding grounds and food supply should have provided more fly, only the shade was insufficient.

So long as there is adequate shade along the water side it does not matter to *Glossina* if the whole of the centre of the island is open grass land. This is the condition on both Bulago and Kiuwa, each of them having above the average number of *Glossina*.

On the other hand Ngamba, forested all over, is below the average. Other islands like Sanga and those of the Dwanga group are also well covered with forest, and have few or no fly, but the presence of large numbers of the huge spiders and absence of good breeding grounds are disturbing factors.

On islands of fair size where the shade along the shore is variable in amount, *Glossina* may be found at points where, if that was the degree of shade on all parts of the island, it would be unable to hold its own.

This is the case on the far western end of Nsadzi, where the line of bushes around the coast is practically only one bush wide, and yet *Glossina* was found there by Mr. Fiske, and by myself subsequently. That is to say, that with a favourable base the fly can extend its range into, and at any rate visit, localities where it probably could not live permanently.

The most favourable shade is produced by two kinds of bushes,

already alluded to by the native names of "*Oluzibaziba*" and "*E'kinsambwe*." The former has been described: the latter has branching stems of very soft wood, and rough hairy leaves, with spikes of inconspicuous yellow flowers furnishing spiky burrs. This shrub is able to exist, like the *Luzibaziba*, on every variety of soil, and is found indiscriminately along sandy, pebbly, marshy or rocky shores.

Thickets of these shrubs, if not *too dense*, afford admirable shelter for the fly. But if they are too thick, they are not so favourable; this appeared to be the case on Kiuwa.

A plant of very different type, with straight reed-like stems bearing large alternating leaves, has been described as "*E'tungulu*." Where this does not grow too thickly it makes admirable shelter for the fly, but if very dense appears to be unfavourable for the same reason that *Papyrus* is. The same may be said of cane thickets, for though cane itself does not throw dense shade, the requisite amount of shade is provided by the tangled creepers always found among cane, such as *Ipomaea* and Papilionaceous species.

Where the cane is very dense, and poorly infested with fly as on Lukalu, they take advantage of the tunnels made by the frequent passage of Hippopotami along the same route.

On entering such a tunnel on Lukalu I was greeted with the buzzing of a small cloud of *Glossina* which were resting there. A forest of tall trees, without much undergrowth, is only productive of *Glossina* in any quantity at its edge, either along the water's margin or, if a narrow strip bordering water, on its landward side, as on the shore of the north bay of Bulago.

Indeed it may be said to be characteristic of *Glossina palpalis* to be found in greatest numbers at the edge of open spaces backed by, or surrounded by, forest. The areas on Tavu where fly was found to be so abundant, are of this nature.

It has been very frequently observed how, when one gets into the forest away from the edge, the numbers of *Glossina* diminish. This is very noticeable on Damba, Tavu, and Wema.

This is not merely due to the fact that one thus gets further away from the water, because on Bugalla in 1912 the same thing was noted in the strip of forest along the coast if one entered it from the landward side, the flies being more abundant at the edge than when one had penetrated a little way.

TOPOGRAPHY.—D. CHARACTER OF COAST.

This factor is intimately bound up with the shade factor. A bare, rocky coast, whether of flat rock gradually shelving into water, or of irregular boulders, is of course unfavourable. What may be termed an "open" coast, such as a beach of sand or gravel backed by shade, is the most favourable for *Glossina*. The "grazing grounds" often alluded to are also very much frequented by the fly.

A shore along which the forest comes as an impenetrable wall to the water's edge, often with *Papyrus* and ambatch in front of it, does not offer the best conditions for the fly.

The question of the character of the coast line, however, is so closely bound up with the question of breeding grounds, that this will be considered next.

TOPOGRAPHY.—E. AMOUNT AND CHARACTER OF BREEDING GROUNDS.

By "Breeding ground" is meant an area of sand, gravel, or fine light soil, dry, and well shaded, near to the water, where the conditions, so far as we at present know them, appear to be favourable for the life of pupæ, which are to be found there in considerable numbers; although sometimes, if the area is large and universally suitable, it may be difficult to find many pupæ at a time owing to their being generally diffused.

Such small localities as hollows at the base of a tree where half a dozen pupæ may sometimes be found, can hardly be termed Breeding grounds; I would suggest for these the term "*Pupal loci*."

I have hitherto assumed that breeding grounds could always be seen from the water, by a survey of the coast from a canoe, but the recent tour produced ample proof that one must make an inspection of the shore on foot; if necessary cutting one's way through the dense vegetation.

Many times good breeding grounds have been found thus, in the ridge of sand or fine pebbles thrown up when the lake was at a former higher level, but now less close to the present coast line, which may be of rocks, or marshy. In many cases one would have said, when surveying the coast from the water, that there was no breeding ground there.

Tavu was a conspicuous instance of this. The W. coast, at about the centre of the island, is either marshy or formed of flat red ironstone rock, on which grows dense vegetation completely hiding a sand bank some twenty yards back from the water and a few feet above it.

BREEDING GROUNDS.

In this sand bank, sheltered by thick bushes, more pupæ were found per boy per hour than in any other breeding ground examined. The work done in association with Mr. Fiske made it clear that the best breeding grounds are, undoubtedly, such as that on Tavu; banks of sand or gravel which formed the beaches of earlier days when the lake was at a higher level, and now usually covered with bushes providing excellent shade, and by reason of their structure well drained; although near to the water not at the actual water's edge.

Of such a type were also the excellent breeding grounds on Wema, Damba and Yempata, Kimmi, Bulago (on the N. point and the western end of the S.W. shore) and the fly-beach on Nsadzi. The various kinds of soil in which, other requirements being suitable, pupæ may be found may be listed as follows:—

I. PEBBLES.

On Yempata, on the W. shore, an excellent breeding ground was afforded by a pebble beach, the pebbles being of small size, and pupæ were collected in this at the rate of 56 per boy per hour.

On Ngamba, Mr. Fiske found a "locus" for pupæ among much larger pebbles, amongst which was vegetable débris derived mainly from ferns. This was the habitual resting place of a *Varanus*, and probably owing to the constant pre-

sence of the lizard the dead fern leaves had been broken up so as to provide a suitable medium for pupæ. Elsewhere, close by, none were found.

II. PEBBLES MIXED WITH COARSE SAND.

This appears to be the most suitable medium for pupæ, and formed the breeding grounds on Wema, Damba, and Tavu, all three very typical. On Ngamba, Lukalu, Marida this type of breeding ground was also present, but the infestation by fly of these islands was small.

III. COARSE BROWN SAND (the detritus of ironstone rock).

This formed breeding grounds on Kerenge, Sindiro, the north side of Nsadzi and the north coast of Bulago. Pupæ have never been found in this in such abundance as in II.

IV. VERY FINE WHITE SAND.

This forms the breeding ground on Bugalla (see second report), and at one time I considered it to be the most suitable medium for pupæ.

I believe now that it lies too closely to be the most favourable medium, and is apt to hold moisture too much.

But there are large beaches of it and pupæ may always be found in it where it is well shaded and dry enough. Breeding grounds of this type occurred on the south point of Bulago and at the middle of the south-western shore, in the south bay of Nsadzi, on the south side of Dyavodemi, and the south point of Yempata. It is a curious fact that all these areas of fine white sand are on the *south* side of the islands. On Masovwi, however, the white sand was on the northern side, and there is some on the north shore of Nsadzi, though not sufficiently shaded to form breeding grounds.

V. FINE LIGHT SOIL.

This often nourishes beds of E'tungulu, which provides the necessary shade. The breeding grounds on the south of Kerenge, on Ziro, and part of the shore of the north bay of Bulago, are of this type.

VI. VEGETABLE HUMUS AND FINE DEBRIS.

This may provide "loci," but is never in sufficient quantity and dry enough to form regular breeding grounds.

On Tavu, for instance, under the shade of a large Luzibaziba bush, was a heap of vegetable dust produced by the burrowing of large carpenter bees into a dead branch, up against which this dust was piled. In this Mr. Fiske found a few pupæ.

On Kiuwa, during a prolonged search, I found a few pupæ in a nook among rocks at the base of a small tree where was a small collection of vegetable débris.

On Lulu such "loci" were the only possible places for pupæ, and also on the eastern forested end of Nsadzi, which is rocky and has no breeding ground. My 9 boys found at one spot where flat rocks were covered with fine rootlets and dry

débris about an inch thick, 8 pupæ and 33 shells in half an hour. This locality was first found by Mr. Fiske when I was not with him. Subsequently I was endeavouring to find a "locus" along this coast, and hit on the same spot, as I afterwards learnt from one of the boys who had been with Mr. Fiske previously.

On Ngamba, under a ledge of rock in the forest sheltering vegetable humus and débris a few empty shells were found, but they had the appearance of having been there a long while, and no fresh pupæ were found.

Loci of this character are important, however, because they can maintain a certain number of flies in the absence of regular breeding grounds.

Similar loci are provided by the bases of large trees in the forest, which at some point have decayed slightly, forming a little hollow arching over dry débris. Such places are often inhabited by ant-lions, which indicate the suitability also for *Glossina* pupæ.

I have found pupæ thus in several places in the forset of the N. shore of Bulago, which is well provided with breeding grounds, and also the fly boys found empty shells in such a locality on the south shore of Ngamba where were no breeding grounds. The practical importance of these scattered loci is obviously very great.

VII. VERY FINE, DRY DUST.

This is found on the floor of caves and under large, overhanging rocks. It is very little used by *Glossina* as a breeding ground, but on Sege a large rock seemed to provide at its base the only possible place for pupæ, and one live pupa and a few empty shells were found here.

On Kimmi, the caves, with large areas of sandy floor in places covered by this dry dust seem to be used very little indeed as breeding grounds. When I first saw the caves I expected that very large numbers of pupæ would be found there, seeing how very abundant was *Glossina* on the island, but no more than 6·7 living pupæ could be found per boy per hour.

On Waitwe the caves formed the only breeding ground on the island, and the only puparia found on the island were here.

On Kiuwa there was a cave on the south shore whose floor was thickly covered with this dust, and though the fly was fairly abundant in the locality, no trace of pupæ or empty puparia was found in the cave.

The dust in these situations appears to be mainly formed by the disintegration of the dried droppings of bats, which frequent such places.

Pupæ have not been found among dead leaves or rotting wood only. A thin layer of dead leaves over the surface of the soil appears to be no hindrance to the deposition of larvæ. Indeed I have somewhat altered my opinion on this matter; formerly I believed that the drier was the medium the better it suited the pupæ, provided they were near to the water so that the atmosphere was humid. Constantly, however, one finds in a large area that

there are certain particular spots in which pupæ are collected closely together. This is often under the shelter of a stem of a small patch of dead leaves, and I now believe that a certain small degree of humidity is requisite, and that that particular spot sheltered by the dead leaves supplies it. Whether this be the explanation or not, it is a fact that in a large breeding ground pupæ often are found collected together in a few square inches, and it is very difficult to say why that one small area is more suitable than the surrounding parts.

I have previously laid stress on the *importance of free air circulation* over the surface of a breeding ground (see my first report, vol. xii., Reports of the Sleeping Sickness Commission, p. 96.) Recent observations on Tavu have furnished very strong support in favour of this opinion.

Beyond the end of the ridge of sand which forms the breeding ground there is more sand, lying at a lower level, and so densely covered with bushes and low plants that the surface of the sand is not exposed, being thickly covered with dead leaves and débris which have a damp, mouldy smell (see fig: 3). This is not the kind of locality where one expects to find pupæ, and a search by the fly-boys did not reveal any. The process of hunting for them, however, cleared away the top layer of dead leaves, etc., leaving, at one place, an area about eighteen inches square, still well shaded by vegetation arching over it, but with the sand left bare.

Here, a week later, I myself found in a few minutes about two dozen freshly deposited pupæ, but there were none in the immediate neighbourhood where the surface was still thickly covered with dead and mouldy leaves.

I believe that the apparently suitable soil on Kiuwa, where we expected to find pupæ and did not, was rendered unsuitable because there was a very thick layer of damp leaves from the Kinsambwe bushes lying on top and effectually preventing aeration of the sand.

A prime requisite for a breeding ground is that it should be well shaded. The shade may be (*a*) permanent, or (*b*) variable.

PERMANENT SHADE

is produced by

- (*a*) Caves, or large rocks undercut at their bases.
- (*b*) Prostrate tree trunks arching over the ground.
- (*c*) Hollows at the base of, or among roots of, living trees.
- (*d*) Thick bushes.

These localities have already been sufficiently alluded to. The best example of (*a*) was the rock on Wema; of (*b*) the breeding grounds on Damba and Bugalla described in previous reports furnish good examples; examples of (*c*) have been described as occurring on Bulago and Ngamba; and (*d*) is the most usual type of breeding ground.

It was a matter of great surprise that the caves on Kimmi did not yield more living pupæ, for very large numbers of empty shells can always be found there. These, however, probably represent the accumulations of ages, for in such places where they are not disintegrated by weather the resistant puparia may probably last for an indefinite time, so that the proportion of pupæ to empty puparia in such localities is much lower than in others. Mr. Fiske pointed out to me that on Wema where pupæ were found in much higher proportion, these places were within reach

of waves during a storm when the water rose high on the beach, and the accumulated shells would be broken up or washed away.

VARIABLE SHADE.

Pupæ and shells may be also found in places where the shade is not always uniform. Thus on the south point of Bulago among the cane and Etungulu thickets ramble papilionaceous creepers which often produce a thick tangle at certain points, weighing down the stems on which they climb so as to form a thick mass of vegetation arching over the ground and throwing dense shade on to it. Here pupæ will be found collected. But these creepers are not always in the same condition, and when their fresh growth dies down in drier seasons spots previously shaded become more exposed to sun.

A plant which, when in full growth produces thick beds throwing excellent shade, is a thick leaved aromatic labiate, with spikes of purple flowers, which grows best in light, sandy soils, to a height of three feet or sometimes more.

Pupæ may constantly be found under the shelter provided by thick beds of this herb, and in places that have often been a surprise.

On Kerenge, for instance, Mr. Fiske found pupæ among coarse sand on top of ironstone slabs where shade was produced by this herb alone. One frequently finds empty shells under clumps of this plant which are at the present time in very poor condition and throwing very thing and inadequate shade. In this connection may be mentioned the correspondence that has been found to exist between the condition in which variable shade occurred at the time pupæ were searched for, and the condition of the pupæ. This bears out in a striking manner the ill effects of exposure upon pupæ, which appear to be killed through insufficient shade. The stages of development of a pupa, as they appear to the naked eye on opening the puparium, may be classified as four.

- I. "*Creamy*."—In which the histolysis that occurred at the end of the larval stage has reduced the contents of the puparium to a cream.
- II. "*Eyes white*."—Where the appendages have been laid down, but the eyes have not darkened.
- III. "*Eyes pink*."—The first indication of the acquisition of colour. From pinkish yellow the eyes gradually acquire their normal colour, when the next stage is reached.
- IV. "*Colour of adult*."—When the eyes are dark and the colour of the bristles of the integument is obvious.
- V. "*Ready to emerge*" with limbs free and Ptilinum extruded.

For practical purposes 1 and 2 were amalgamated, as it was found often impossible to say to which class a freshly deposited pupa should be ascribed.

Classes 1, 2, 3 are grouped as "early" stages; 4 and 5 as "late" or "advanced."

I am indebted to Mr. Fiske for demonstrating these stages to me.

The percentage of pupæ in the early stages has been found on many occasions to be much greater in localities where there was

a thick green growth of vegetation of recent development. Two localities on Wema may be taken as a contrast, visited both on February 26th. At one place a very thick tangle of young green shoots of a species of *Polygonaceae* grew on a ridge on the fly-beach and arched over the surface below. A fly-boy found here 88 pupæ, of which 68 were creamy, 12 with pink eyes, only 8 in colour of adult stage, and none ready to emerge. That is to say, roughly, 91% had been recently deposited, the growth of young, green shoots having only recently become dense enough to induce the flies to take advantage of its shelter. Moreover, only 6 empty shells were found at the same time. Contrast this with what was found at the undercut base of the rock previously described, which was only a few hundred yards away on the same stretch of shore.

In this locality, where the shade conditions would be always the same, out of about 260 pupæ found, 35.4% were advanced, and 64.6% early.

As it is probable that stages 1, 2, 3 take up a much greater part of pupal life than 4 and 5, these proportions are what might be expected in a locality where larvæ are constantly being deposited, on the assumption that a pupa takes about three weeks to develop.

In this locality there were twice as many empty shells as pupæ.

As regards diminution of shade causing death of pupæ the following observations are to the point.

On the fly-beach of Nsadzi Isle on February 3rd one boy found a localised collection of pupæ and shells, obtaining 41 shells and 9 pupæ. These latter were *all dead*. On inspection of the locality we saw that the shade there was less than it had been, the herbs at the edge of the clump having died, but further towards the middle of the thicker vegetation there were living pupæ.

On Kisigalla, on February 11th, I found an overhanging rock sheltering some light, dry soil. Some creepers hung down in front of the nook so formed, but were dead, and at that time threw no shade. In the loose soil were some ant-lion pits, and two *Glossina* pupæ lay on top. They were quite near the pits, and it seemed as if the ant-lions in throwing out the earth to make their pits had also thrown out the pupæ buried therein, which had then lain exposed on the top, and, owing to the death of the creepers, had been killed by sun which could now penetrate into the nook.

On Bulago, on March 12th, a boy searched for pupæ at the base of a small Muvuvumye tree, a thinly foliated, papilionaceous tree which throws but little shade (a species of *Smithia*). This one had recently died, and the plants growing round its base were also thin and poor, though they had evidently at one time thrown good shade. Here 132 shells were found and 5 pupæ, all dead.

Pupæ were several times searched for on Bulago in the E'tungulu bed on the shore of the north bay. The first search considerably damaged the E'tungulu, so that much of it died.

When pupæ were looked for later in the same places, under the dead or dying E'tungulu which now threw very poor shade, 15 out of 18 pupæ found were in the advanced stages, that is, the flies were no longer depositing pupæ here since the shade had become insufficient.

On March 14th, on another part of Bulago, pupæ were searched for under some large, horizontal branches of another Muvuvumye tree, which was in very poor condition and threw but little shade.

Arching over these branches, which were near to the ground, was a thin growth of half-dead vegetation, forming a canopy which, however, did not, at the time, throw dense shade, though formerly it probably had done so. There were some new young shoots growing up. Now, out of 45 pupæ found here, 27 were dead, 15 creamy, and 3 colour of adult. I believe these facts can be thus explained. The herbage having died and throwing shade insufficient to protect the pupæ that had been deposited, they subsequently died. Now, however, that the young growth was beginning to thicken, the shade was better, and flies were again depositing larvæ, so that the living pupæ were found to be nearly all creamy. It thus appears that localities may be suitable for pupæ at one time and not at another. Indeed one might suppose that the places that have been called permanent breeding grounds might serve for the fly in dry weather when variable shade was at its minimum, and that in wetter times when vegetation was more exuberant fewer pupæ in proportion would be found in the permanent localities, since other places would also be suitable. The caves at Kimmi should afford proof of this, and will be visited regularly for this purpose.

The question must now be considered. What effect has the presence or absence of a good breeding ground upon the numbers of adult flies, other conditions being favourable? In other words, are the flies especially numerous on certain islands because the conditions are eminently suitable for their pupæ; or are pupæ to be found in numbers because the conditions are particularly favourable to the adult fly? In the light of present knowledge I believe the facts to be as follows: *Glossina palpalis* is able to exist in numbers about the average (which at the time of our tour in January, February, March, 1914, was found to be represented by a catch of 13.1 ♂♂ per boy per hour) even in the absence of any good breeding grounds as we know them.

Kiuwa and Lula may be considered in this connection. On Kiuwa, for the whole island, the average number of males caught per boy per hour was 15.1. Now it has been pointed out what extraordinary difficulty we had on Kiuwa in finding pupæ. What we at first thought would prove to be breeding grounds of good quality were not then being used by the fly, for reasons which I have given, and we could only find a few pupæ here and there among rocks.

On Lula there were no breeding grounds at all, and the number of flies (represented by 6.8 ♂♂ per boy hour) was apparently kept up by pupæ deposited in vegetable débris in sheltered nooks in the rocks. Again, on the eastern forested end of Nsadzi there are no breeding grounds. The number of flies in the forest at the time we visited this island was represented by 12 ♂♂ per boy per hour, and a few pupæ have been found in a thin layer of vegetable débris and fine rootlets lying on a slab of flat rock.

Now the question may at once be asked, if such nooks among rocks are sufficient to maintain *Glossina* in such numbers as have been given above, why can they not find similar nooks on other rocky islands, where food and shelter are both adequate, such as Rumfua, and those of the Dwanga group, where, as a matter of fact, there were found no fly?

It is an interesting fact that on all these islands there were the huge spiders in great abundance, so that they, and not the absence of breeding ground, may account for the absence of fly.

In this connection a comparison between Rumfua islet and the adjacent shore of its large neighbour Kome (of which it is structurally a portion) is of great interest. Rumfua has already been described as a rocky island, without any breeding grounds, but well covered with shade producing vegetation; it abounds to an extraordinary degree in the large spiders, and has no *Glossina*.

The adjacent part of the shore of Kome is bold and rocky and well covered by shade-producing vegetation, as on Rumfua, though a little further away the coast is bare, with bold rocks and cliffs. There is no breeding ground on that part of Kome adjacent to Rumfua, and along the whole stretch of its coast from a point on the N. shore opposite to Rumfua, around the W. end of the island, and along the S. coast as far as Rumfua, I have only found two places suitable for pupæ. The one on the N. coast would certainly have nothing to do with the flies on the S. coast, the two coasts being separated by intervening hills without shade. The one place on the S. coast where pupa were found in numbers sufficient to enable it to be called a breeding ground was a sandy bight, between which and the coast opposite Rumfua was a long stretch of bold, rocky shore without any shade. So that it is almost certain that the flies which were found on that part of Kome opposite Rumfua, to the number of 5·7 ♂♂ per boy per hour, deposited their pupæ in isolated nooks among rocks, at the base of trees, and so on, where, indeed, one or two were actually found. So then on that part of Kome *Glossina* exists under the same general conditions as are found on Rumfua, with the single exception that Rumfua has the large spiders in great abundance whereas Kome has them in very small numbers. So that it seems that the spiders on Rumfua and the other islands, and not absence of breeding grounds, are responsible for absence of fly. On Lula, where there were no big spiders, absence of breeding grounds did not prevent the fly having a certain footing. *I desire, therefore, to lay stress on this important conclusion, that the fly can maintain itself in certain numbers without any good breeding ground.* If, however, such breeding grounds are present, then the numbers of the fly rise considerably to an extent the check on which is not known. *On all islands with an infestation above the average degree such good breeding grounds have been found, and no island has been found without good breeding grounds on which infestation by Glossina was above the average.* Moreover, those islands that had good breeding grounds, well dispersed, and on which the fly was below the average number, had some other factor that may account for the low degree of infestation. Thus, Sindiro was a spider island, so were Kerenge, Ziro, Marida, and Dyavodemi. Sindiro was not very much below the average, and, as has been said, the spiders were at one end of the island in the old plantations. All these islands had good breeding grounds. As regards Kibibi, which was only a little below the average, it may be said that the breeding grounds were confined to the western half of the N. shore, and their limitation may explain the low degree of infestation on an island that seemed in other ways very favourable. Kizima had abundance of good breeding ground, but the sum total of shade seemed insufficient. Lukalu, which had excellent breeding grounds of large extent, had a low degree of infestation which is more difficult to explain than the other cases. It may be that the total amount of shade in proportion to the size of the island was insufficient, for although the

S. and N.E. shores had good shade for the greater part of their extent, yet the rest of the island was devoid of shade.

The question whether the neighbourhood of a breeding ground has any effect upon the proportions of the sexes of the fly in that locality cannot be answered definitely. On some islands the flies caught in the neighbourhood of a breeding ground showed a higher percentage of females than elsewhere, but on other islands the reverse was the case. It appears to be more likely that in a locality where food is easy of access the females are caught in a lower percentage than elsewhere, and this will be discussed under the heading Food Supply.

INTER-RELATION WITH OTHER ANIMALS.

Glossina must, like every other living thing, have very complicated relations with its organic environment. Inasmuch as my knowledge of botany is insufficient to enable me to discuss the relation between *Glossina* and the vegetation amongst which it lives, I propose to discuss here only its relations with other forms of animal life, leaving out the question of animals in the aspect of food supply, which will be discussed separately.

In the first place it may be said that it does not seem probable that the unusual abundance of *Glossina* on certain islands is to be explained by "insularity," that is, the limited amount of competition owing to fewness of species, as is to be explained, for instance, the abundance of the beautiful blue Hesperid butterfly on Sanga, or the two species of beetles noted on Mtiomu. These, and other insular peculiarities, are noted in the general account given of each island at the commencement of this report. Also, there seems to be no relation at all between the general suitability of an island for insect life, as evidenced by the number of species of butterflies to be found on it, and the degree of infestation by *Glossina*. Table I. shows the number of butterflies, and this should be compared with column 1, in Table II. Neither could any relation be made out between the numbers on any island of the species of different families of butterflies, and the infestation by *Glossina*; that is, it does not appear that the prevalence of any one group of butterflies indicates an environment also particularly suited to *Glossina*.

As regards other insects mention has been made in the description of each island of any notable feature. Insects such as might act as *enemies* of the adult fly were met with on three islands.

On Nsadzi, the colony of *Bembex forcipata*, alluded to in the first report, was found active in March of this year, but not in January when the first visit was made. An individual took a *Glossina* from off the clothes of one of us while the fly-boys' catch was being counted.

A large colony of *Bembex* was also noted on the sandy S. point of Bulago; these individuals were active in January. Seeing how numerous are the flies in this locality the balance that has been struck between *Bembex* and *Glossina* is not unfavourable to the latter.

Dyavodemi was remarkable for the great abundance of large *Asilidae*. A species of Asilid was recorded in my Second Report to have been seen to catch a *Glossina*; but none have been seen that select *Glossina* as a specific prey.

SPIDERS.

The large spiders (? *Nephila*) form such a feature of the fauna of many islands that they require special mention. On the map those islands heavily infested with them have their names underlined in black. It is seen that of islands close together some may be spider islands, and others not. Thus Wema and Marida were heavily infested, while Yempata (lying between Wema and Sindiro, both infested) was free, as also were the three islets close to Marida. Lula was free while all the other small islets off the eastern end of Kome were heavily infested.

None of the group around the western end of Kome were infested (save Rumfua, which is rather isolated), although individual spiders were seen on Kibibi, Kiuwa, Tavu, and Bulago of the same type that formed such an infestation elsewhere.

On most of the infested islands the spiders seemed to find ideal conditions in old banana plantations, now overgrown with jungle. Indeed, with the exception of Rumfua (where there were no plantations), and Kisigalla and Sanga (both small islands on which the plantations had been completely overwhelmed) the spiders might be said to be practically confined to such localities. Here the sheets of webs may be seen in planes one behind another with scarcely a foot of space in between, and in such extraordinary numbers that the spiders on the central webs can scarcely get enough food; indeed from the pinched appearance of the abdomen they must suffer considerable deprivations. An individual web may measure several feet, and be strong and sticky enough to entangle birds—in fact a sunbird was found on Dwanga Mto hopelessly entangled. The fact that abundance of spiders is correlated as a rule (Wema being an exception) with few *Glossina* has been mentioned already, and the question arises: Does predominance of the spider act as a check upon *Glossina*?

Mr. Fiske, on several occasions on Wema, found *Glossina* in the webs, and the spiders greedily ate such *Glossina* as were given them. A complication is introduced by the fact that on many spider-islands breeding grounds are so deficient that their absence may be the factor reducing the numbers of the fly, e.g., Dwanga Mkuru, Dwanga Mto, Dwasendwe, Rumfua, Sanga. On other islands; however, there are good breeding grounds associated with abundance of spiders. On Wema, for instance, at least on that part of it examined, the breeding grounds were so uniformly excellent that the presence of spiders in great abundance seemed to be unable to check *Glossina*. It must be said, however, that only at some points along the shore where the overgrown banana plantations, which are the favourite haunt of the spider, came very close to the fly-beach, did large numbers of fly and spider coincide. It has been previously pointed out how the numbers of *Glossina* diminished on Wema a little way back from the shore—whether in a spider-haunted plantation or in the pure forest where spiders were practically absent.

On Sindiro, where the number of flies was just below the average, the breeding ground, though good, was all at one end, the S. point. Spiders abounded here, as also on the N. shore at a point where there were remains of old plantations, but no breeding grounds.

On Kerenge, Dyavodemi, and Ziro, the old plantations scattered about the island were in each case the main home of the spider;

the breeding grounds in each case were apparently of good enough quality to have provided more flies than were actually found, so that it seems as if on these islands the spiders actually keep down the fly. On Marida there were superb breeding grounds on the isthmus, but they were, however, apparently unable to produce enough flies to overcome the destruction by the spider, which abounded in the same locality.

On Kisigalla there was perhaps enough breeding ground to account for more flies than were found, if the similar, localized, and relatively small breeding grounds on Ngamba, where fly was more abundant be borne in mind. The spider, therefore, introduces a complication in the presence of breeding grounds; that is to say, if the assumption is correct that it has a limiting effect upon the numbers of the fly. On this assumption, large extent of excellent breeding ground on Marida is unable to produce enough flies to overcome destruction by the spider; on Wema the influence of very fine breeding grounds more than counterbalances destruction by spider; on other islands abundance of spider and absence of breeding grounds go hand in hand with absence of *Glossina*. The combinations of conditions that may be found can be thus arranged:—

Condition.	Fly.	Spider in quantity.	Excellent breeding grounds.
1	+	+	+
2	±	+	+
3	+	—	+
4	+	+	—
5	±	—	—
6	±	—	+
7	—	+	—

Of condition 1 Wema is an excellent example.

The typical condition 2 would have fly altogether absent, but no such example was found. Marida, on which fly was much below the average (indicated by sign $\pm$) comes under this grouping.

Of condition 3, Tavu is a typical example.

No example of 4 has been met with, but could one be found, *i.e.*, an island with spider and fly both abundant, but no good breeding ground, it would show the unimportance of the former as a check, and that breeding grounds are not a necessity.

Of condition 5 no typical example has been found—that is, a locality where fly is abundant in absence of both spider and breeding grounds; but Lula, as it had *some* flies, is an incomplete example.

Lukalu, where the infestation was not very much below the average, is a fair example of condition 6.

Condition 7 is well illustrated by Rumfua.

An interesting zoological feature of such islands as were particularly infested by the spider was the absence of small birds such as frequent undergrowth and bushes; and, often, a compensatory abundance of predaceous insects (*Mantidae*, and on one island, *Asilidae*). It would be a difficult question to decide whether the primary absence of birds permitted great increase of the spider, or whether, spiders being abundant, the small birds

disliked the risk of getting entangled in the webs, or found insufficient food, and left the locality. The latter alternative would hardly seem to be the true explanation, for on many spider-islands insects (as exemplified by butterflies) were unusually abundant.

ENEMIES OF PUPÆ.

In my first report it was mentioned that pupæ may be found the contents of which have been devoured by some arthropod that had bitten a hole through the shell, usually near the base of the two bosses. The size and shape of such a hole varies enormously; sometimes it is relatively small; at others nearly half of the pupal case has been eaten away. A complication is introduced by the fact that sometimes *empty* puparia from which the fly has emerged (as shown by the typical splitting of the apex) have been found bitten into in this manner. A practice was made of collecting empty puparia as well as living pupæ, and it was found that the number that had been bitten into varied enormously. No pupa was counted as "eaten" unless the apical end was intact, showing that the imago had not emerged. From the accompanying table the proportion of "eaten" pupæ to every 100 puparia from which the fly had emerged is indicated. It was highest on Nsadzi, and next highest on Damba; but persistent search on Nsadzi failed to reveal the creature that had done the damage.

TABLE SHOWING PROPORTION OF "EATEN" PUPÆ.

Locality.	Empty puparia collected.	Living pupæ collected.	Number of eaten pupæ in proportion to every 100 puparia
Nsadzi	417	53	20.6
Damba	3,939	797	14.6
Sindiri	91	9	4.4
Kerenge	179	60	3.3
Wema	1,436	1 048	2.
Kibibi	225	56	1.3
Tavu.. ..	578	489	1.2
Kizima	84	10	1.
Kimmi	520	69	0.9
Bulago	2,213	207	0.7
Lukalu	31	5	0.
Kiuwa	148	15	0.

The fact that on Nsadzi apparently one-fifth of the pupæ are destroyed must not be thought too much of, seeing that on Damba where *Glossina* was much above the average in numbers, about one-seventh were similarly destroyed.

In most localities where any quantity of pupæ are collected one may occasionally find a pupa that has been parasitised, showing the small, round hole of exit of the parasites (*Chalcididae*) described in my first report.

On Wema, owing to the systematic opening of every puparium obtained, we discovered several that were full of parasitic larvæ, and by collecting more pupæ from the same locality were able to rear the adult parasites, specimens of which accompany this report. These have been described as a new species (*Syntomosphy-*

rum glossinae)* but subsequently have been proved to be parasitic not on the *Glossina*, but on a species of *Mutilla* which itself destroys *Glossina*. These *Chalcididae* are therefore favourable to *Glossina*.

FOOD SUPPLY.

The question of food supply as a factor controlling the fly is fitly considered here next to the relation of the fly to other animals. Allusion has been already made to Mr. Fiske's suggestion that the proportion of *female* flies caught by the boys depends upon the readiness with which they come to feed; that both sexes are really present in equal numbers but the more active and curious males come first and are more readily caught, while as a general rule only those females that are hungry come to be caught. This theory receives support from the following facts'—

1. The experiment, already described, of catching those flies only which settled on the conspicuous white net, and then those only which were desirous of feeding.
2. Certain fly-boys, who are more expert at catching flies from off their clothes, invariably catch a greater proportion of females. One boy who caught flies from off an open black umbrella set upon the ground, always caught more females than the other boys. The umbrella appeared to the flies to be something from which they could feed, as they could be seen vigorously probing it with their proboscides. Similarly, more females can be caught from off the dark sides of a canoe drawn up on the beach. These facts seem to indicate that the fly finds its food as much by sight as by any other sense.
3. The remarkable coincidence between the degree to which one is bitten on any particular island and the proportion of females in the catch; in fact the order of precedence is the same. On Kimmi we were more viciously attacked by flies than on any other island; they came off in a swarm to the canoe even before we had got to the shore. Wema and Yempata came next in order of viciousness of the flies.

On Damba, however, where, judging by the males, the flies were nearly as numerous as on Kimmi, they gave very little trouble, and the percentage of females caught was only a quarter of that on Kimmi (see Table II.). At present, therefore, it seems as if the percentage of females caught is an indication of the greater or less degree of accessibility of food supply. In certain cases this accessibility is manifest.

Thus, when we visited Tavu, in January, it was more frequented by crocodiles than any other island investigated. I counted nine off the coast when I came round it in the canoe. The percentage of females caught was a little below the average level and, judging by the males, flies were more numerous than on any other island.

Again, on Ngamba island, six crocodiles were counted during the circumnavigation, and the percentage of female flies caught was here lower than on any other island visited. The large water

*Footnote.—Bulletin of Entomological Research, March, 1915. Vol. V., part 4, pp. 365—370.

frequenting lizard, *Varanus*, was not abundant on either island.

Now, on the other hand, when we visited Kimmi no crocodiles were seen and *Varanus* was very scarce, only one being met with.

On Kimmi the female percentage was highest of all.

The islands which appeared, from a single visit, to be most frequented by *Varanus* are not heavily infested by *Glossina* (cp. Table 1 and 2). This may be explained, perhaps, by the habits of *Varanus*, which seems to prefer rather rocky, open shores on which to bask, and such basking places may often be recognised. On Kizima, for instance, there were very many, although only two lizards were actually seen at the time of our visit; on Kizima there were very few flies.

Rumfua, whose bold, rocky shores offered ideal basking places for *Varanus*, had no *Glossina*, and on a single visit five *Varanus* were counted on the rocks at the water's edge.

Lukalu is also very much frequented by *Varanus*, five having been seen, and the infestation by *Glossina* was below the average.

These facts may suggest that abundance of food does not imply abundance of fly, but it must be remembered that it is just the localities most frequented by *Varanus* for basking in the sun that are least suitable for *Glossina*, either from lack of sand for breeding in, or from lack of shade.

Mention may here be made of another point, while discussing *Varanus*. If the columns of Table 1, showing numbers of *Varanus* and crocodiles on each island, be compared, it will be seen that, generally speaking, islands most frequented by crocodiles are least frequented by *Varanus*, and vice versa.

For instance, Tavu, Ngamba, and Kiuwa, which head the list with crocodiles, are very low down in the *Varanus* column, while on Rumfua, Ziro, and Lukalu, which were favourite resorts of *Varanus*, no crocodiles were seen.

This does not appear to be explicable by choice of different kinds of shore. It is true that on Tavu the greater part of the shore is marshy and fringed with rushes, which is not the locality most beloved of *Varanus*, but on Ngamba and Kiuwa the crocodiles frequent just the rocky type of shore which is the favourite resort of *Varanus*.

Is there then some natural antipathy between the two reptiles? It is well known that *Varanus* devours the eggs of crocodiles, and the crocodile would seem to be aware of this, from the fact that it returns to the nest to keep watch over the eggs. Two of the three localities where we found crocodile's eggs bore on the sand the imprint of the ventral plates of the crocodile. It is probable, indeed, that the depopulation of the islands has resulted in an increase in numbers of *Varanus*, and a corresponding decrease in crocodiles. For the Baganda very eagerly hunt *Varanus* to obtain the skins for drum heads, and since their removal from the lake shore *Varanus* must have increased enormously. On the other hand, I have often been struck by the scarcity of crocodiles in the lake, at any rate that part of the lake among the islands that I have explored. Considering that there is nothing, save *Varanus*, to harm them now that mankind is out of the way, and that the lake abounds with large fish, one would have expected to see many more crocodiles than is actually the case.

Now if it be true that the accessibility of food is indicated by the proportion of female flies caught, then on islands where

the percentage of females varies greatly, it should be possible to point out in some localities more food supply than at others. The islands will now be considered in this way.

On *Bulago* the greatest ♀ %age when we visited it in January was on the S. point. At that time there were no crocodiles frequenting it, nor were *Varanus* seen there, although hippopotami were frequently seen in the water close by. The lowest ♀ % was at the N. shore, near to the forest and to the N. point, where *Varanus* was often seen. This locality was also frequented by hippopotami.

On *Nsadzi* the greatest ♀ %age was on the N. side of the island. Neither crocodiles nor *Varanus* were seen here, whereas both frequented the S. bay, where the ♀ %age was lower. Hippopotami were seen in both localities.

On *Kiuwa* the highest ♀ %age was in the old plantations where no *Varanus* were met with, and the lowest %age on the S. coast where several crocodiles and *Varanus* were seen.

On *Kibibi* the greatest ♀ %age was at the N.E. point, which was not thought to be a suitable locality for either reptile, whereas a crocodile was seen in the S. bay and several *Varanus* near by on the S. shore, the ♀ %age in the S. bay being the lowest on the island.

Ngamba does not quite follow the rest of the islands considered; the greatest ♀ %age was on the N. shore and N.W. point. Here, however, crocodiles and hippos were seen. The lowest ♀ %age was on the S.E. point and E. shore; the former was frequented by *Varanus*, and on the latter it was evident that Sitatunga antelope had been feeding on the bushes.

Ziro had the greatest percentage of females in the old plantations on the N.E. side, where no *Varanus* were seen; the lowest ♀ %age was at the S.E. point where the lizards were numerous.

On *Kerenge* the ♀ %age was the highest in the W. bay where were seen no *Varanus* or crocodiles, nor did it seem a likely locality for them. At the entrance to the E. bay, two *Varanus* were seen, the percentage of females was lowest.

On *Sindiro* there was a lower percentage of females at the S. point, which was much frequented by *Varanus*, than at the W. coast of the island, which did not seem suitable for them.

It will have been noticed in the preceding paragraphs that the presence or absence of Hippopotami does not seem to affect the ♀ %age, implying that these animals are of little importance as a source of food. That the flies do feed on them was stated in my second report, where I gave an account of having actually witnessed them doing so.

But there is little doubt of the relatively far greater attractiveness to *Glossina palpalis* of reptilian blood. Emphasis has been already laid upon the importance of *Varanus* as a food supply in previous reports, and it has been thoroughly established by an experiment carried out on Kimmi during our tour, at Mr. Fiske's suggestion.

On January 26th, a live *Varanus* was tethered at a place where flies were very numerous and hungry, and close to it a couple of goats, one completely black, the other black and white. In the first hour of the experiment it was estimated that some 70—90 flies fed from the *Varanus* and that 22 went to it for every 1 that went to the goats. As many as 30 could be seen feeding from it at once.

When the *Varanus* was allowed to lie at the edge of the lake in shallow water with only part of the back and top of the head exposed (as it frequently does in the wild state), flies persistently settled and bit the exposed parts, returning again and again as often as they were washed off by wavelets.

On January 29th, a similar experiment was performed with a young live crocodile, of almost the same size as the *Varanus*. One of us counted every fly that fed fully, none being counted until it had been seen to extrude the superfluous fluid, so that many which fed only partially are not included. The other observer recorded the fly and site where it fed as announced. Between 8.35—9.45 a.m., 40 flies were noted to have fed to repletion, favourite sites being the neck and neighbourhood of eyes and nostrils; but the flies were able to penetrate between the plates of back and legs.

RECORD OF FLIES BITING CAPTIVE CROCODILE ON KIMMI
—JANUARY 29th.

Time a.m.	Site chosen.
8.35	?
8.45	?
8.55	Eyelid
8.56	"
8.57	"
8.57	"
8.58	Flank
8.59	Neck
8.59	"
9	Eyelid
9.2	Shoulder
9.2	Hind leg
9.6	"
9.6	"
9.6	"
9.7	Eyelid
9.7	Hind leg
9.8	Eyelid
9.9	"
9.10	Neck
9.10	Leg
9.10	Nostril
9.11	Eyelid
9.18	Angle of mouth
9.18	Eyelid
9.23	Hind leg
9.25	Neck
9.27	"
9.30	Flank
9.31	Hind leg
9.35	Neck
9.36	Fore leg
9.36	"
9.37	Eyelid
9.38	"
9.42	Nose
9.42	Hind foot
9.43	Nose
9.43	Hind leg
9.45	Back
Total 40 flies.	

As regards the potential source of mammalian food, on most of the islands it is composed of Hippopotami, otters, and bats. On a few islands small rodents are very abundant in the long grass, but here they are out of reach of the fly which does not venture out on to the grassy hills.

On only the largest islands (Kome, Damba, Wema) were monkeys found, and Sitatunga only on Kome, Damba, Yempata, Nsadzi, and Ngamba, though they may only *visit* the last two from Kome. The Sitatunga, where it occurs, does seem to be an important source of food (see my second report, Reports of the Sleeping Sickness Commission, vol. 14, pp. 20).

But the Hippopotamus seems almost negligible as is shown by the following recent work done on Kome and the six small islands off its west extremity.

On every occasion when these small islands have been visited, since the end of April, the flies which showed that they had recently fed were brought home to Kome to be examined, and the character of the blood was noted. Flies have also been collected on the shores of Kome, and similarly examined. Now the mammalian fauna of the smaller islands only differs from the fauna of Kome in the absence of monkeys and Sitatunga—that is so far as can be ascertained. Hippos., bats, and otters occur on them all, and rodents on some in similar numbers to those of Kome. If, then, there is any difference in the proportions of non-mammalian (N) to mammalian (M) blood from these various localities, it must be due to the Sitatunga (for monkeys are negligible) on Kome, or the lack of reptile food on Kome. *Hippopotami are equally accessible on Kome and the smaller islands.*

Below are given the figures up to date of writing.

Island.	Flies examined containing recent blood.	M %age.	N %age.
Kome	85	33.0	67.0
Bulago	40	5.0	95.0
Tavu.. ..	41	4.9	95.1
Kizima	12	0.0	100.0
Nsadzi	31	6.5	93.5
Ngamba	62	6.5	93.5
Kimmi	74	6.8	93.2

The above figures show well the overwhelming importance of reptilian food, even on Kome, where the Sitatunga antelope is responsible for the mammalian blood found in the flies examined. Kizima also shows that the mammalian blood in Kome flies is not derived from the rodents which inhabit the thick grass tussocks in great numbers, for they are equally abundant on Kizima.

As regards Ngamba the mammalian blood may on this island as well as Kome have come from Sitatunga, for they certainly at least *visit* the island even if they do not live there, and the same may be said of Nsadzi.

Kimmi is particularly frequented by Hippopotami, which have their regular sleeping places in the forest, and are constantly to be seen in the water off the coast, sometimes as many as five together. The arguments used against Hippopotami being fed

upon to any great extent apply equally to bats, which abound on the small islands. On one occasion we met with an enormous concourse of large fruit bats on Ngamba, and having caught one I hung it in a bag of mosquito net in a locality where flies were plentiful; its warm body pressed closely against the net, but for the hour during which I watched not a single *Glossina* paid any attention to it. In regard to otters as a food supply the same arguments apply.

Since, then, crocodiles and *Varanus* are such an important source of food for *Glossina palpalis* their habits are worth considering. If they only frequent certain islands at certain times for the purpose of oviposition, for instance, then there must be great variations in the supply of food for the fly of those localities. Thus, on Kimmi, the large numbers of holes dug by *Varanus* in the open sandy area of the E. coast, together with remains of eggshells found there, indicate that the lizard goes there in large numbers to lay its eggs. But not more than two at any one time have yet been met with on Kimmi, where they are decidedly scarce.

The season at which crocodiles oviposit would seem to be, from the present evidence, about the end of the year. The crocodile requires an open bed of dry sand, not too conspicuous, but hidden by bushes growing around it. During our tour among the islands three nests of eggs were found in such localities, on Marida, February 21st; on Masovwi, March 6th; and on Kizima, March 11th.

On Marida there was very evident the imprint of the crocodile's body plates on the sand over the eggs, of which there were 55. The embryos were fully formed and seemed almost ready to hatch.

On Masovwi 59 eggs were found containing embryos fully formed but only one-third of the size of those on Marida.

On Kizima again there was the imprint of the crocodile's body on the sand. Here 60 eggs were found, containing embryos of about the same size as those on Masovwi.

According to Gadow (Camb. Nat. Hist., vol. Reptiles, p. 465) the embryo is fully formed at the age of eight weeks, but requires another month's growth before hatching. The eggs on Masovwi and Kizima were, then, probably laid early in January, those on Marida about the end of November previously. As it seems, from the imprints of the mother's form on the sand over the eggs, that she returns there from time to time and lies there, she must afford ample opportunity to *Glossina* to feed upon her.

I may say here that quite recently I have been able to approach so closely to a full grown crocodile lying inertly in the bush of Ngamba, that I was able to see quite plainly two *Glossina* on it, and one on the side of the neck was most certainly feeding. So that the time of oviposition of the crocodile, and some time afterwards, will mean for the *Glossina* of certain islands an abundance of food. Since this should enable the female flies to produce larvæ at a more rapid rate, the number of larvæ deposited, and of pupæ to be found, should be greater at such times, and should result in a few weeks in increased abundance of flies.

Thus, on an island where there was found to be a high percentage of females it might be supposed that reproduction was not so active as on another island where the percentage of females caught was much lower.

In order to bring into relation the number of females caught and the number of pupæ found, I have adopted here my former plan (see p. 11 of my second report) of dividing the number of pupæ found per boy per hour by the number of females caught per boy per hour. The resulting figure, called empirically "Pupæ per female" gives a means of comparing one island with another. The following table shows well the *inverse* relation between the female percentage and "Pupæ per female" figures. In compiling the table those localities only were taken in which sufficient pupæ were found for a fair estimate to be made, and the figure for female percentage is from that locality only.

TABLE III., SHOWING RELATION BETWEEN ♀ %AGE AND REPRODUCTION.

♀ %age.		"Pupæ per female."	
Kimmi (whole) .	.. 53.1	27.1	Tavu (W.)
Yempata (S. & W.)	.. 37.5	6.2	Damba (1.3)
Kibibi (N.) . . .	.. 37.1	3.	Bulago (S.)
Wema (3.4.5.6)	.. 37.	2.4	Wema (3.4.5.6)
Kerenge (E.) ..	.. 28.2	2.3	Kerenge (E.)
Nsadzi (S.) ..	.. 16.1	2.1	Nsadzi (S.)
Ngamba (N.) ..	.. 15.	1.4	Yempata (S. & W.)
Bulago (S.) ..	.. 14.	1.2	Kibibi (N.)
Damba (1.3) .	.. 10.5	1.	Ngamba
Tavu (W.) ..	.. 5.7	0.2	Kimmi
Average	.. 25.4	4.7	Average

It is very clear from the above figures that the extremes are inverse, those islands that head one column being at the base of the other. No island is either at the head or the base of both columns. These facts, therefore, amply support the theory.

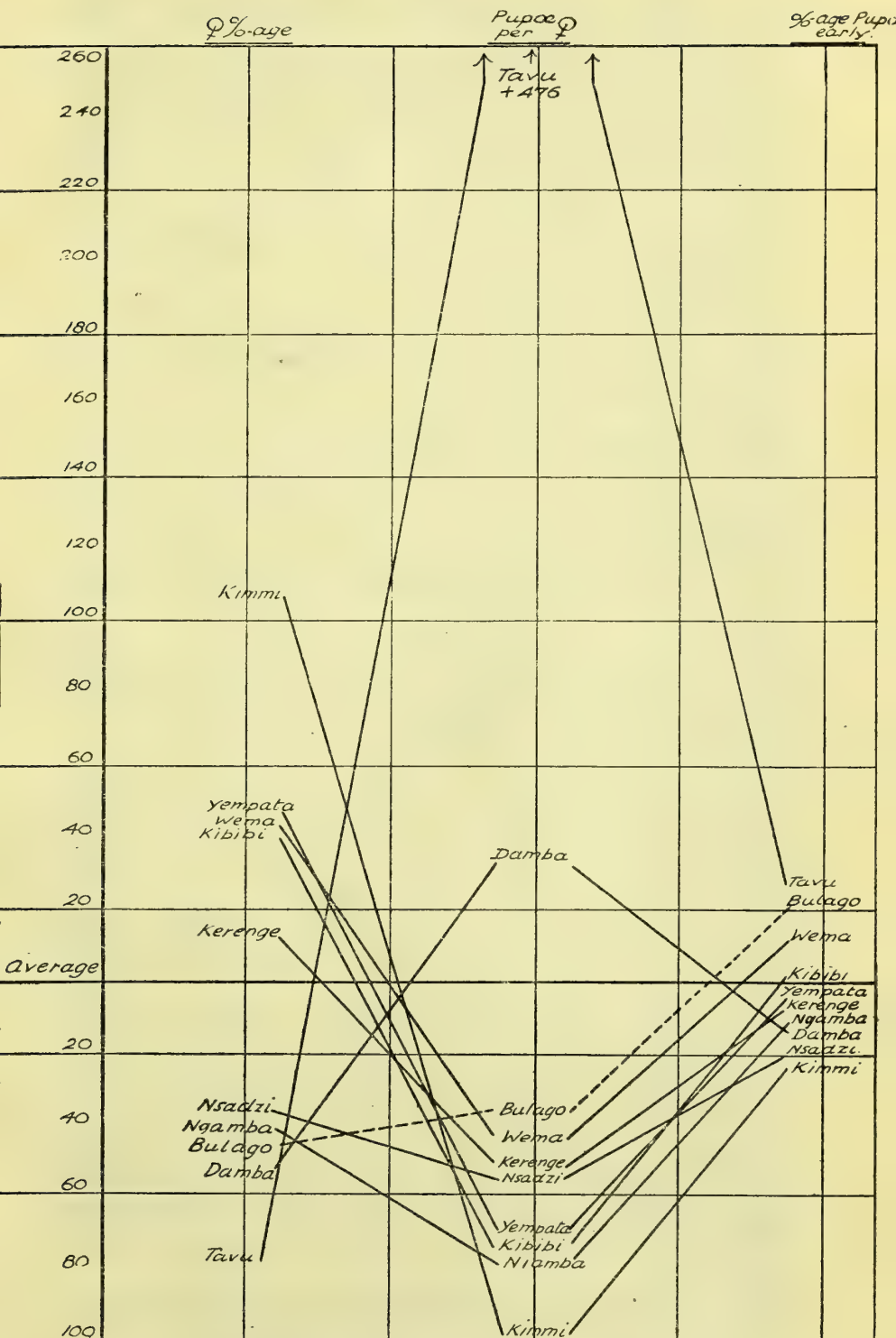
The relationship comes out even more clearly if the amount per cent. that each island is above or below the average for each column is plotted as in Table IV.

The case of Tavu requires a word of explanation. It will be noted that its "Pupæ per female" figure is 476% above the average! This extremely high figure is explained by the relatively small area of the breeding ground, so that very large numbers of pupæ could be found concentrated in it in a very short while.

In the cases of Damba, Yempata, Wema, and Kimmi the breeding grounds were very much more extensive, and pupæ presumably more generally distributed.

It might be argued that if on Kimmi the number of pupæ found per boy per hour is small because the pupæ are scattered, and large on Tavu because they are concentrated, then the position of these islands in the table is explained without any reference to the percentage of females. But it has been pointed out that pupæ were also scattered over large areas on Damba and Yempata, and yet the inverse relation between the two columns is just as true as is the case with Tavu and Kimmi. The principle that has been discussed can be developed still further. If at one locality reproduction is active and large numbers of larvæ are being deposited, *i.e.*, if the "Pupæ per female" figure is high,

TABLE IV.



then the pupæ, on being examined, should be found to be mostly in the early stages of development. The different stages have been already alluded to.

On Table IV. I have plotted out, simultaneously with the two records previously discussed, the percentage of "early" pupæ for the same localities; as in the other cases taking the average as the base line and expressing the figures as percentages above or below the average. There is less variation than in the other columns, but one could hardly expect the proportions of pupæ in various stages of development to vary very greatly.

The curves so formed on the chart show that when the percentage of female flies is high, the percentage of pupæ found in the early stages is low, and vice versa.

Further, if the female percentage being high indicates that reproduction is active, then a large number of pupæ per female should be associated with a figure for percentage of early pupæ above the average, and vice versa. Tavu and Kimmi, for example, represent exactly opposite conditions.

All the curves, save one, on the table agree, in that their *concavities* are, as in the cases of Tavu and Kimmi, towards the base line: if the extremes are above the average, the middle is below, and vice versa. The single exception, which is not a marked one, is Bulago.

If, then, the correct explanation of a high percentage of females in the catch be scarcity of food supply, and if this reacts unfavourably on the rate of reproduction, it cannot be expected that the flies should remain on Kimmi in the state in which we found them, great numbers with high percentage of females. So that according to Mr. Fiske's theory, the numbers at least must decline, unless the food supply becomes more readily accessible, in which case the female percentage should fall.

The suggestion is that the varying facilities for obtaining food must reflect themselves in the numbers of the fly and the percentage of females caught.

During the present year it is my purpose to carry on further work upon this point upon the six islands in the neighbourhood of the W. end of Kome, to ascertain if the variations in the numbers of flies do accord with the theory.

One would expect that if the reptiles furnishing the food collected at one island, they must deprive the flies on others, so that on the former the flies should vary in a manner precisely the opposite of that on the latter.

In conclusion it may be said that at present there are grounds for believing that the accessibility of *reptilian* food supply is an important factor in regulating the numbers of *Glossina palpalis* on the islands of small size, and that mammalian blood is of little importance save on the larger inhabited by antelope.

A few words may now be said about *Climate* as a factor controlling the fly. It must be at once admitted that my previous suggestion cannot be upheld, namely, that the low percentage of females on the islands is due to their greater susceptibility to higher temperatures (see second report, p. 10). For on islands as close together as Tavu and Kimmi the proportions differed enormously.

Seeing how close together are many islands differing greatly in the manner of their infestation the effect of climate as a factor controlling *Glossina* differentially may be disregarded.

A greater degree of rainfall might influence islands on different parts of the lake, but not so close together as those that have been considered. The degree of exposure to winds from the lake has some effect on the thickness of the vegetation, and this was very noticeable on the three islands of the Dwanga group, where the exposed eastern sides of the islands showed the vegetation to be checked, the trees being stunted and not of such luxuriant growth as on the S.W. side, sheltered by Kome. This would probably have some effect on the distribution of the flies on such islands.

Finally, let us try to arrive at some conclusion as to the reason why certain islands head the list as regards infestation by *Glossina*. Taking the seven which stand well above the average at the top of the list we find they agree in the following points:—

1. Breeding grounds of excellent quality, nearly always the ridges of sand or gravel thrown up by the lake at a former higher level, and now left dry.
2. Abundant good shade, with, however, open spaces to give the fly scope for wandering.
3. Absence of marked infestation by the large spiders. The apparent exceptions of Wema and Sindiro have been dealt with.

If we leave out, for the present, Kiuwa and Sindiro, which are respectively next above and below the average, the following table can be made for the remaining islands.

TABLE V.

Island.	Breeding ground factor.	Shade factor.	Absence of spider factor.
Kibibi	—	+	+
Lukalut†	+	—?	+
Ngamba†	—?	—	+
Dyavodemi	+	+	—
Lula	—	+	+
Ziro	+	+	—
Marida	+	+	—
Kerenge	+	+	—
Sege	—	—	+
Kizima	+	—	+
Masovwi†	+	+	+
Kisigalla†	—?	+	—
Waitwe	—	—	+
Wavuziwa	—	—	+
Sanga	—	+	—
Dwanga Mto	—	+	—
Dwanga Mkuru	—	+	—
Dwasendwe	—	+	—
Rumfua	—	+	—
Isentwa	—	—	+

† It is a little doubtful if the factor queried is really deficient.

‡ Masovwi is so small that, as in the case of the seven islets which come below it in size (Table I.), that alone may account for scarcity or absence of fly.

Thus, in all the islands below the average (except Sindiro) there is lacking one, or more of the three factors all present on every island (save Kiuwa) that is above the average.

It is worth noting that in the eight islands at the bottom of this list (arranged in order of decreasing infestation by *Glossina*) two factors are absent, whereas of the ten islands at the top of the list only one (Sege, which is lowest but one, and only has one example with one factor lacking between it and those with two lacking) has more than one factor lacking.

This, in itself, strengthens the statement that these three factors must all be present to ensure abundance of fly well above the average.

In three islands was the breeding ground factor alone lacking.

In two islands was the shade factor alone lacking.

In four islands was the absence of spider factor alone lacking.

In four islands were the breeding ground and shade factors lacking.

In five islands were the breeding and absence of spider factors lacking.

In one island all factors were present, but its small size was probably responsible for fewness of fly.

Finally, what is the value of the observations and conclusions here reported in helping us to get rid of *Glossina palpalis*? This is to be done, theoretically, by

1. Clearing, to drive away adult fly.
2. Clearing, to destroy breeding grounds.
3. Systematically catching adult fly.
4. Systematically collecting pupæ.
5. Starvation of fly.
6. Infecting fly with disease-producing organisms such as *Nosema apis*.
7. Introduction or encouragement of enemies.

1. The work here reported on does not make it any more likely that clearing need be *less* extensive than has always been considered necessary. The S. shore of Nsadzi, where fly is able to exist along very thinly shaded, or even bare, shore by virtue of better shelter in other parts, shows that clearing must needs be very complete.

2. I have already emphasized the fact that the fly can exist without definite breeding grounds. Therefore, although clearing of these alone might lessen the numbers of the fly it would not exterminate it.

3. Catching the adult fly destroys *females* in small proportion only on most of the islands. Such a method as the sticky cloths on animals, devised by Maldonado, would be preferable to hand-catching in any locality such as the islands where males predominate in the catch.

4. Collecting pupæ. I am inclined to think that, done as suggested below, this would be the most effective way of reducing numbers; for it must be remembered that *half* of the pupæ are females. There is near Mpumu a sandy beach on the mainland where, formerly, pupæ were collected in numbers for laboratory purposes. But after some time, by about 1910, they were to be

found in such small quantity that the locality was no longer used. My recollection of the locality is faint, but I think it was a stretch of good breeding ground along an otherwise unfavourable shore. Now on Damba, which was next used as a source for pupæ, constant collection had no effect, but *there were other* long stretches of good breeding ground close by. The ideal thing would be to clear all breeding grounds, and provide here and there *artificial shelters* of a form agreeing with shelters found to be naturally chosen by the fly.

4. These could be kept under control, and if the fly could be enticed to larviposit there, rather than in scattered nooks and crannies, they could be cleared of pupæ at regular intervals, which need not be shorter than a fortnight or perhaps even three weeks. I am at present carrying on work on these lines.
5. Attempts to starve the fly by extermination of large mammals would have no effect upon *Glossina palpalis*, which naturally prefers reptilian blood. It would be a difficult matter to destroy all crocodiles, *Varanus*, and other large reptiles.
6. As regards infecting the fly with disease I still hold this to be impracticable. Even if a few flies were infected artificially, the peculiarly specialized mode of life is against the infection being communicated to other flies through soiled food, as is the case with *Nosema apis*. It is true that I was in error, in writing in my second report (q.v., p. 19) that the fly does not void any superfluous fluid until it has finished feeding and flown away. When watching them feed on the crocodile and *Varanus* tethered on Kimmi isle, I saw that they do exude fluid before withdrawing the proboscis, presumably to make room for more blood. But the chances of another fly accidentally inserting its proboscis through a drop of such fluid which has dried on the skin are remote, especially as all infectious material would be washed off when the crocodile or *Varanus* went into the water. This mode of infection could not apply in the case of a hairy animal, since the fluid exuded would be caught by the hairs and would not rest upon the skin so that the fly might by chance insert its proboscis through a layer of infective material.
7. Such natural enemies of *Glossina* as exist have already struck a balance with it, as would presumably any new foe that might be introduced.

G. D. HALE CARPENTER.

June, 1914.

APPENDIX TO THIRD REPORT.

FURTHER OBSERVATIONS ON THE REPTILIAN FOOD
SUPPLY OF *G. PALPALIS*.

Since the third Report was written I have by chance made three interesting observations on the food supply of *Glossina*.

On Ngamba island, on May 25th, I came unexpectedly and suddenly upon a large crocodile lying among the bushes on a narrow peninsula. It was apparently sick, as it had a wound at the base of the tail and did not run away at my approach. By watching within a few feet I was able to see two *Glossina* on its neck, and one was certainly feeding. I may say that flies are extremely scarce at that part of the island. I believe this to be the first time that wild flies have been seen feeding on a crocodile under natural conditions.

On the same island, a species of black snake, with yellow markings on the ventral surface of the neck, is very abundant.

On June 27th, I came upon one of these, in length about 3 feet, in the blind and lethargic condition previous to casting the skin. It paid no attention to me, so that I was able to sit down beside it on the rocks, close to the water. I had seen two *Glossina* sitting on it as I came up, and during the half-hour for which I watched there were two flies obviously attracted by the snake, which moved a little at intervals. The flies were both males, and neither of them made attempts to feed from either the snake or myself. But they frequently settled on its neck, and were quite evidently attracted by it.

I have no doubt whatever that had I come upon a snake in this condition on Kimmi in January, when the flies were so hungry and the female percentage so high, I should have seen many flies feeding on the snake.

On Bulago Island, on June 26th, I saw a *Varanus* with many flies on its back, some of which had the swollen bodies indicating that they had fed. This is now the second occasion this year, and the sixth during the whole of my investigations, on which I have seen numbers of *Glossina* on a wild *Varanus*. So that I have ample evidence of their liking for *Varanus*, and have seen them feeding on a crocodile, and attracted by a tortoise (on Bugalla), and a snake (on Ngamba).

G. D. HALE CARPENTER.

June 30th, 1914.

On July 6th, 1914, I saw a python lying on the shore of Kimmi and two *Glossina* were on it, one of which had certainly fed.

G. D. HALE CARPENTER.



FIG. 1. The raised beach on Ngamba cleared of vegetation up to the edge of the forest behind. Two boys are searching for pupæ under the shelter.



FIG. 2. The shelter on the raised beach of Ngamba, after clearing.

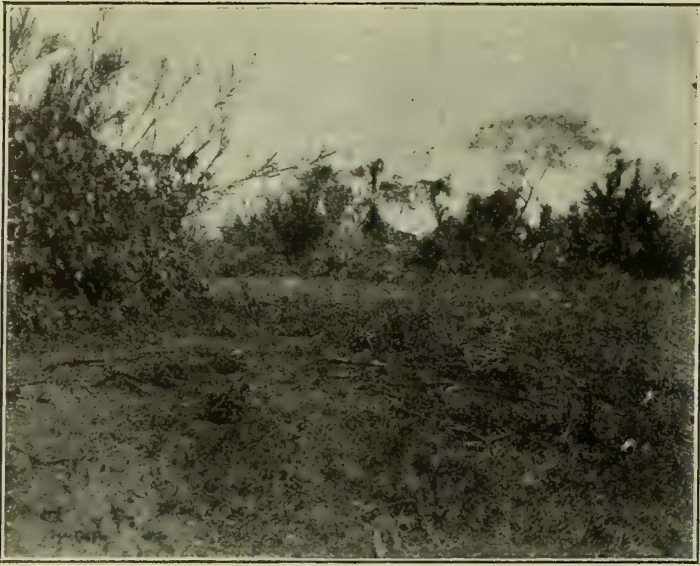


FIG. 3. Raised beach on Tavu after clearing. The highest point of the ridge (which makes a right-angled turn) is at the centre of the picture. The vegetation formerly covering the ridge was like that on the left; this shelters sand too damp for pupæ, so that it was not necessary to cut it. (See p. 45.)



FIG. 4. The shelter on the raised beach of Tavu, after clearing. The water is to beyond the reeds on the right hand.

FOURTH REPORT ON THE BIONOMICS OF
GLOSSINA PALPALIS.

ON SHORES OF L. VICTORIA.

This report deals with work done between the end of April and middle of August, 1914.

Owing to my services being required for military duties the work came to an end, suddenly, and there was no opportunity of writing up results until a year later, when I availed myself of a fortnight's leave at Entebbe. The report deals with the following questions:—

- 1.—Experiments with artificial breeding grounds.
- 2.—The relation between Speke's Tragelaph and *T. gambiense*.
- 3.—Variations in numbers of the fly.
- 4.—For how long can the fly live under starvation?
- 5.—Does the fly circulate round an island?
- 6.—The stages of development of the pupa.

EXPERIMENTS WITH ARTIFICIAL BREEDING
GROUNDS.

In my last report it was stated that *G. palpalis* (*fuscipes*) can exist in numbers up to the average, represented by 13 ♂ per boy hour, on the islands examined, even in the absence of any good breeding ground; but that if good breeding grounds are present the numbers of *Glossina* may rise considerably, to an extent limited only by checks whose nature is not apparent.

It is significant that no island has been found *without* good breeding grounds on which the fly was above the average in number, and on the other hand those islands that had good breeding grounds, well dispersed, and on which the fly was *below* the average in number, had some other factor that might have accounted for the small number.

When these facts are considered it appears that local clearing of good breeding grounds may succeed in keeping down the numbers of the fly, but will not wholly exterminate them.

But if the fly could be enticed to make use of some easily controlled, artificial breeding ground, in which the pupæ could be readily collected at regular intervals, providing much more favourable conditions than scattered nooks and crannies, so that they would be actually *attracted* to come there for larviposition, a combination of local clearing and artificial breeding grounds might succeed in very considerably reducing their numbers. It must be remembered that *half* of the pupæ are females; whereas the organised catching of adult flies does not destroy nearly so many females as males.

At the close of my tour amongst the islands between Entebbe and Jinja, which formed the subject of my last report, I decided to undertake an experimental test of the value of this method on Ngamba Island, lying S.E. of Entebbe, between the larger islands of Kome and Nsadzi, which is particularly well suited for such an experiment (see map, p. 76).

Only at one part of the coast line is there a good breeding ground (the N.W. corner), elsewhere the shore is rocky or marshy. This breeding ground is, as usual, a sandy beach left by the recession of the lake, and on it grew numbers of shrubs and herbs,

with masses of *Ipomæa* creeper, forming abundance of good shade. The infestation on this island was moderate; the maximum number that has ever been caught there being represented by 45.4 ♂ per boy hour, and the average for all localities for the period April 20th—August 24th being 26.4 per boy hour.

By the end of April, 1914, I had got this breeding ground quite cleared of the sheltering vegetation, and had made an artificial shelter on the cleared sand. This was formed of a sloping, thatched roof about twelve feet long and four feet broad; its lower side about a foot and a half above ground, its higher side about three feet above the ground, and the sand beneath it being cleared of all vegetation. This was made on April 21st (see figs. 1, 2, above).

I had previously set the boys to hunt for pupæ under the sheltering herbage, but they had only found twenty-three, which were either dead, parasitised, or half eaten by some predaceous insect. This gave a rate of 3.2 per boy hour, which was a higher figure than had ever before been obtained on Ngamba.

Empty puparia were found abundantly, at the rate of 116 per boy hour.

On May 9th, four pupæ were found under the shelter; they were in the "creamy" state, that is, they were freshly deposited.

On May 16th, no more were found.

On May 25th, nine boys in half-an-hour found thirty pupæ, of which twenty-six were in the creamy condition, three with eyes pink, and one dead. This gave a rate of 6.7 per boy hour, *i.e., more than twice as many as had ever been found under natural conditions.* This result is made more striking if one remembers that the pupæ that were found before the ground was cleared represented an accumulation of an indefinite period, limited only by the duration of the pupal stage, whereas the above figure represents only nine days.

On June 1st, seven boys in half-an-hour found thirteen pupæ, nine of which were "creamy" and four had the adult colouring. These latter had probably been overlooked on a previous occasion, as they would not have reached this stage of development in so short a time.

On June 8th, nine boys in half-an-hour found only seven pupæ, of which six were creamy and one dead.

On June 15th, ten boys found only three pupæ. Two of these were in the site that had been covered by a dead leaf lying on the surface. This fact suggested that the flies preferred the sand to be lightly covered rather than bare; indeed, I wrote in the last report that a thin layer of dead leaves appears to be no hindrance and a certain small amount of dampness in the soil a requisite. In order to test this a thin layer of dead leaves was scattered over one-half of the sheltered sand.

On June 20th, only two pupæ were found, but both were in that half where the dead leaves covered the sand. On this day the leaves were put on the other half of the area, and that half first covered was left bare.

On June 27th, only two pupæ were found, but they were again both in the half covered by dead leaves, though this time it was the half which had been bare on the last occasion. This is sufficient to show that the fly prefers the sand thinly strewn with dead leaves, to a bare surface.

The next step was to strew the whole surface with dead leaves.

This at once proved of value, for on the next visit on July 4th, eight pupæ were found (2.7 per boy hour); on July 11th, twenty-eight (7 per boy hour); and on July 18th, thirty-eight (9.5 per boy hour).

A test was then made whether dead leaves or logs of wood on the surface would be more attractive, and on July 18th half of the sand was strewn with leaves, and logs were put on the other half. On the 29th there was a drop in the number of pupæ, only three were found in the half with logs, and four in the other half; the numbers are too small to prove anything. On the 15th August, in the part where logs were, four boys found ten pupæ, and in the other part six boys found nine pupæ. On the 24th August no pupæ were found under the logs, and nine were found by eight boys under the leaves. As the total number of pupæ that could be found on Ngamba was always small, it was decided to try the same experiment on Tavu, where fly is very abundant and large numbers of pupæ had been found under natural conditions.

The breeding ground on the E. shore, formed by a long raised beach of sand, measuring about 50×10 yards, was thoroughly cleared of the dense growth of cane and bushes, and a shelter was erected similar to that on Ngamba (see figs. 3, 4, above). But as there were other breeding grounds on the S. and E. sides of the island, which were too big to be cleared by the few men at my disposal, it was not possible to hope that the artificial breeding ground would furnish more pupæ than had been found before it had been cleared, for the fly would presumably still prefer the natural ground that was left. That is, of course, always supposing that the fly roams about seeking for the most suitable spot when the larva is full grown, and not that she makes the best of whatever spot she finds herself to be in when the larva is ready to be dropped. Evidence will be produced that the flies *do* travel along the shore of an island.

It is suggestive that one so often finds collected together numbers of pupæ in one quite small spot, and this raises the question whether there is some chemiotropic influence that leads the pregnant flies to that particular spot—possibly the fact that there is one pupa there already may lead other flies to drop more larvæ in the same spot.

Before the breeding ground on Tavu was cleared, four boys in an hour found 606 pupæ, giving a rate of 151.5 per boy hour. On May 14th, the ground was thoroughly cleared, and a shelter erected as on Ngamba; and a fortnight later 10 boys found 107 pupæ under the shelter in 40 minutes, giving a rate of 16 per boy hour. Of these pupæ, 56 were examined, and 53 were found in the creamy state; some had been so recently deposited that the integument had not yet hardened. On June 3rd, 141 pupæ, all "creamy," except two, were found under the shelter, giving a rate of 28.2 per boy hour. On June 17th, there was a drop, only 10.7 per boy hour being found. On July 1st, the rate was 20.2 per boy hour. Dead leaves were now lightly strewn over half of the bare sand under the shelter, with the result that on the next visit on July 10th pupæ were found at the rate of 51.5 per b.h. (103 by 4 boys) in the leaf covered sand, and at rate of 34.8 per b.h. (87 by 5 boys) in the bare sand. The dead leaves were now strewn over the other half, and the half which had been first covered left bare.

I was not able to look for pupæ there again until July 22nd, when the half with leaves on it provided pupæ at the rate of 27.2 per b.h. (68 by 5 boys), and the bare half at the rate of 12.4 per b.h. (31 by 5 boys). So that there is no doubt that a *surface thinly covered with dead leaves is more attractive to the fly than bare sand.*

On July 30th, nine boys found 175 pupæ (at rate of 29.2 per b.h.) and then half of the sand was strewn with dead leaves, and logs were laid upon the other half. On August 16th, five boys found 147 pupæ in half-an-hour in the log-covered half, and the other five boys found 148 in the same time in the leaf-strewn half, this gave a rate of 59 per b.h. for the whole shelter. There is thus, so far, *no evidence that logs form a more attractive covering on the surface than do dead leaves.*

If it be remembered that the contents of the shelter were cleared out every fortnight, whereas the number found at any time in the natural breeding ground represents an accumulation of several weeks (*i.e.*, the period of the pupal life), the results are encouraging. These experiments were only the first step, but it is clear that the fly can be induced to larviposit under chosen conditions.

Much remains to be done before it can be said that the method is practicable. Besides covering the sand with strewn leaves, I think providing fresh green growth over the shelter would make it more attractive; several examples were given in my last report, showing how one could find large numbers of freshly deposited pupæ under a thick mass of young green foliage. *Ipomaea* creeper was planted and was already showing good growth over the shelters, when all work was brought to an end by the exigencies of military duty.

It still remains to be seen what is the best size and kind of shelter; whether it should face any one particular direction of the compass, what is the best height of roof, etc. It is obvious that so long as the pupæ have to be sought by hand, the roof must be high enough to allow a boy to creep under. But if fine meshed, shallow trays were used, sunk flush with the surface, and filled with the sand, they would only need to be lifted out, when the sand would fall through and the pupæ be left behind. In such a case the covering roof might be only a few inches above the sand, and might provide a still more attractive shelter. If this method of attacking the fly is tried on a large scale, it is necessary to take especial care when clearing a breeding ground that the edge of the herbage that is left is driven sufficiently far back, and does not shelter soil suitable for the pupæ. It must be remembered that a very favourite site for them is at the edge of a strip of thick herbage.

When the first clearing of the breeding ground was done at Tavu it was not carried sufficiently far back, and at the next visit there were found, by 10 boys in half-an-hour, 107 pupæ at the new face of the belt that was left. Of these 107, 42 were dead, 23 of them quite recently dead. These had probably been in the sand before the sheltering vegetation was cut down, and *exposure to sun had killed them.* Of the 65 that were alive, 57 were very fresh ("creamy"), 3 were more developed, and only 5 in an advanced state of development; that is they had almost all been deposited in the very favourable *new breeding ground opened up by insufficient clearing.*

The edge of the vegetation was driven back a bit further by more clearing, so that it covered only low lying, damp soil, and at a subsequent visit no more pupæ were found at the edge. Further illustration of the fact that by disturbing existing conditions one may open up new breeding grounds is given by the following.

Close to the breeding ground on Tavu was more sand, lying at a lower level, and so densely covered with bushes, and dead leaves on the surface, that there was a damp, mouldy smell about it, and no pupæ could be found there. But on a subsequent visit to a spot about two feet in diameter, where the boys had previously cleared away the damp-holding débris when looking for pupæ, so that the underlying sand had been, as it were, aired, I found in a few minutes about two dozen freshly deposited pupæ where there had been none before.

Conclusions.

1.—It is possible to entice *Glossina palpalis* to larviposit under artificial shelter after the breeding ground has been cleared of its sheltering vegetation.

2.—On Ngamba Island more pupæ were found under such a shelter than had been found when the breeding ground was in its natural condition.

3.—The fly much prefers the surface to be lightly covered with dead leaves or logs, but shows no preference for one over the other. Also the shelter itself should be covered with thick, young, green growth.

4.—A combination of clearing of localized breeding grounds with construction of artificial shelters on the cleared areas is worth trying on a larger scale. A lake island offers ideal conditions for this.

SPEKE'S TRAGELAPH AND T. GAMBIENSE.

When Nsadzi Island was visited in January, 1914, I was much interested to see there undoubted tracks of Speke's Tragelaph, or Sitatunga. My canoemen were quite certain that there had never been antelope on the island when it was inhabited, and it is probable that this is the case, for there is only shelter for them on a small part of the island (the forested E. end), where they could very readily have been hunted down.

I saw footprints of a large individual again in June, in the midst of a very small patch of shelter, hardly more than a few bushes, on the very bare and exposed S. shore. Since the large island of Kome, only separated from Nsadzi by two narrow channels with the island of Ngamba in between (see frontispiece), has numbers of the antelope, it is probable that they reached Nsadzi from there, and in the absence of the natives have established themselves there.

Now, it is a striking fact that when experiments were made some years ago to test the infectivity of flies on the islands after the removal of the natives, no infection could be produced in monkeys by feeding on them flies from Nsadzi Island, although elsewhere they were infective.

The following is taken from the twelfth report of the commission, 1912, pp. 71, 72.

(Reports of the Sleeping Sickness Commission of the Royal Society.
No. xii., 1912, pp. 71—72. London: H.M. Stationery Office.

9.—Duration of the Infectivity of the *Glossina palpalis* after the removal of the Lake-Shore Population. By Capt. A. D. Fraser, R.A.M.C., and Dr. H. L. Duke.)

EXPERIMENT 591. MONKEY.

Nsadzi Island.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911			1911.		
Feb. 2 ..	300		Feb. 8 ..	410	
" 3 ..	465		" 9 ..	335	
" 4 ..	450		" 10 ..	280	
" 5 ..	450		Feb. 13—	} —	—
" 6 ..	275		Mar. 1	} —	
" 7 ..	300				

Remarks.—Result negative. From March 1st—March 27th, monkey 591 was examined bi-weekly and trypanosomes were never found in its blood. In this experiment 3,265 flies failed to cause an infection.

EXPERIMENT 592. MONKEY.

To ascertain if *Glossina palpalis* caught on the islands are capable of giving rise to Sleeping Sickness in healthy monkeys.

Nsadzi Island.

Date.	No. of flies put on.	Trypanosomes.	Date.	No. of flies put on.	Trypanosomes.
1911.			1911.		
Feb. 4 ..	450		Feb. 9 ..	335	—
" 5 ..	450		" 10 ..	280	—
" 6 ..	275		Feb. 13—	} —	—
" 7 ..	300		Mar. 3	} —	
" 8 ..	410	—			

Remarks.—Result negative. From March 7th—March 27th, monkey 592 was examined bi-weekly and no trypanosomes were ever detected in its blood. In this experiment 2,500 flies failed to infect a monkey.

It will be seen that 5,765 failed to infect two monkeys some years after the population had been removed.

Having seen the footprints of the antelope on the island I considered it would be worth while to test again the infectivity of the local *Glossina*, and accordingly flies were caught there and fed on a young male monkey which was kept in the Kome Camp quite out of reach of *Glossina*. The flies were caught in the forest on the E. end of the island, which would be the most likely haunt of the antelope.

Date.				No. of flies put on.			Monkey's blood.
				♂	♀	Total.	
May	11	..	..	77	25	102	
"	13	..	..	19	4	23	
"	19	..	..	71	27	98	
"	22	..	..	106	18	124	Tryps. absent
June	2	..	..	139	11	150	" "
"	5	..	..	137	16	153	" "
"	11	..	..	203	6	209	" "
"	16	..	..	193	13	206	" "
"	19	..	..	142	9	151	" "
"	24	..	..	157	5	162	" "
"	29	..	..	135	13	148	" "
July	7	..	..	192	9	201	" "
"	13	..	..	191	14	205	" "
"	23	..	..	—	—	—	" present
				1,893	183	2,076	

No trypanosomes were seen until July 23rd, when several were seen in one film. On July 29th, two only were seen in a stained film. On August 4th, none could be seen in a stained film. The total number of flies put on the monkey was 2,076, that is, less than half of the number of flies that did not produce an infection in February, 1911, produced infection in 1914; and this is associated with the arrival of buck on the island in the continued absence of the population.

In order to make certain that the Trypanosome seen in the monkey was *T. gambiense*, a cage of bred flies was fed on the monkey on July 29th, August 1st, 4th, 8th, 11th, and 15th, and on the 15th put to feed on a goat which was also injected with citrated blood from the monkey.

GOAT EXPERIMENT.

Date.					Blood.
Aug.	15..	..	Flies fed on	..	
"	17..	..			—
"	18..	..	" " " "	..	—
"	20..	..	" " " "	..	—
"	22..	..	" " " "	..	—
"	23..	..			—
"	25..	..	" " " "	..	—

On August 26th, I was called away from the islands for service with troops, and had to leave the goat in charge of a native; it died on December 25th, cause uncertain, which, so far as it goes, suggests that the Trypanosome was *gambiense*. On August 19th, blood from the monkey was injected into one of two white rats. This one, I am informed, died between October 20th and 30th, the control being in good health. This evidence is also in favour of the Trypanosome not being *T. brucei*. This experiment, though not absolutely conclusive, affords very strong evidence

that the *Tragelaph* does introduce infection to flies not hitherto infected.

The importance of the *Tragelaph* as food for *Glossina* has been dwelt upon in my previous reports, and the following figures give further proof of this.

Island.	Dates between • which flies captured.	Mamm. blood.		Non-mamm. blood.		No. of flies in which Flag- ellates seen.
		♂	♀	♂	♀	
Bulago ..	May 1—Aug. 21	4		105	2	3
Ngamba* ..	Apr. 20—July 20	5		95	4	2
Tavu ..	Apr. 22—Aug. 18	5		106	3	5
Kimmi ..	Apr. 6—Aug. 17	4	1	99	13	
Kizima ..	Apr. 24—Aug. 19			16		1
		18	1	421	22	11
Nsadzi ..	May 13—June 10	2		27	2	
Kome (W) ..	May 2—July 31	37	4	88	11	7
		39	4	115	13	7

*As regards Ngamba it must be acknowledged that although the *Sitatunga* has never been actually seen on the island it undoubtedly at least visits it to feed there.

The flies that were examined were picked out from the days' catches, as they appeared to contain blood.

These seven islands are divided into two groups, according to whether the *Sitatunga* is present or not.

On the five not inhabited by the antelope, mammalian blood was found in the proportion of 4%, non-mammalian 96%, or

$$M : N :: 4 : 96.$$

But on Kome and Nsadzi the proportions were

$$M : N :: 25 : 75.$$

Now on all the islands there are bats, otters, and hippos to serve as a source of mammalian blood, but Kome and Nsadzi have also got antelope, and Kome has monkeys. I do not think that the latter are of any importance as food for *Glossina*, so that the difference between the blood content of flies from the two sets of islands must be due to the antelope.

These figures also bring out the fact that hippos are a very unimportant source of food compared to reptiles, for on every island one finds them, and their resting places in the fly-infested forest and bush, and yet on islands where there were no buck, so that the mammalian blood found in flies was presumably derived from hippos, it was only present in 4% of the flies examined.

Conclusions.

1.—Speke's *Tragelaph* has been found on an island where, when the island was inhabited by natives, it was not present.

2.—The *Glossina* on that island now is able to infect a monkey with a Trypanosome, believed to be *gambiense*, which has not previously been obtained from the flies on that island.

3.—The blood of Speke's *Tragelaph* is much preferred by *Glossina* to that of the hippo.

THE VARIATIONS IN NUMBERS OF THE FLY.

The tour among numerous islands at the beginning of 1914, which was the subject of the last report, made it clear that the variations in the numbers of the fly were of great interest; and it was decided to camp on the W. end of Kome Island, and to study over a prolonged period the fluctuations on the small neighbouring islands of Bulago, Tavu, Kimmi, and Ngamba, which could be regularly visited in turn from Kome (*see map, p. 76*). This work was commenced at the end of April, each island, so far as possible, being visited weekly, and flies caught at a number of localities, from which the average for the island was calculated. Unfortunately, at the end of August, I had to leave the island for service with troops.

It became obvious that the numbers of flies on each of the four islands do not vary together, and that variations do not affect both sexes in the same way. The only means of judging the number of flies on the island is by the number that are actually caught, so that, in the case of the female, if it is a fact that they are only caught because they are hungry, it is difficult to estimate the actual number present. It is probable, however, that the number of males caught gives a fair indication.

On Bulago Island, for instance, on the S. point the sexes on the whole varied together, also on the W. shore. On the shore of the N. bay they varied inversely, and there was a tendency to inverse ratio on the shore of the N. point; but in the forest on the N. point the variations of the ♂ were very great, whereas the number of ♀ kept very constant (*see Charts 4, 5, pp. 80, 81*).

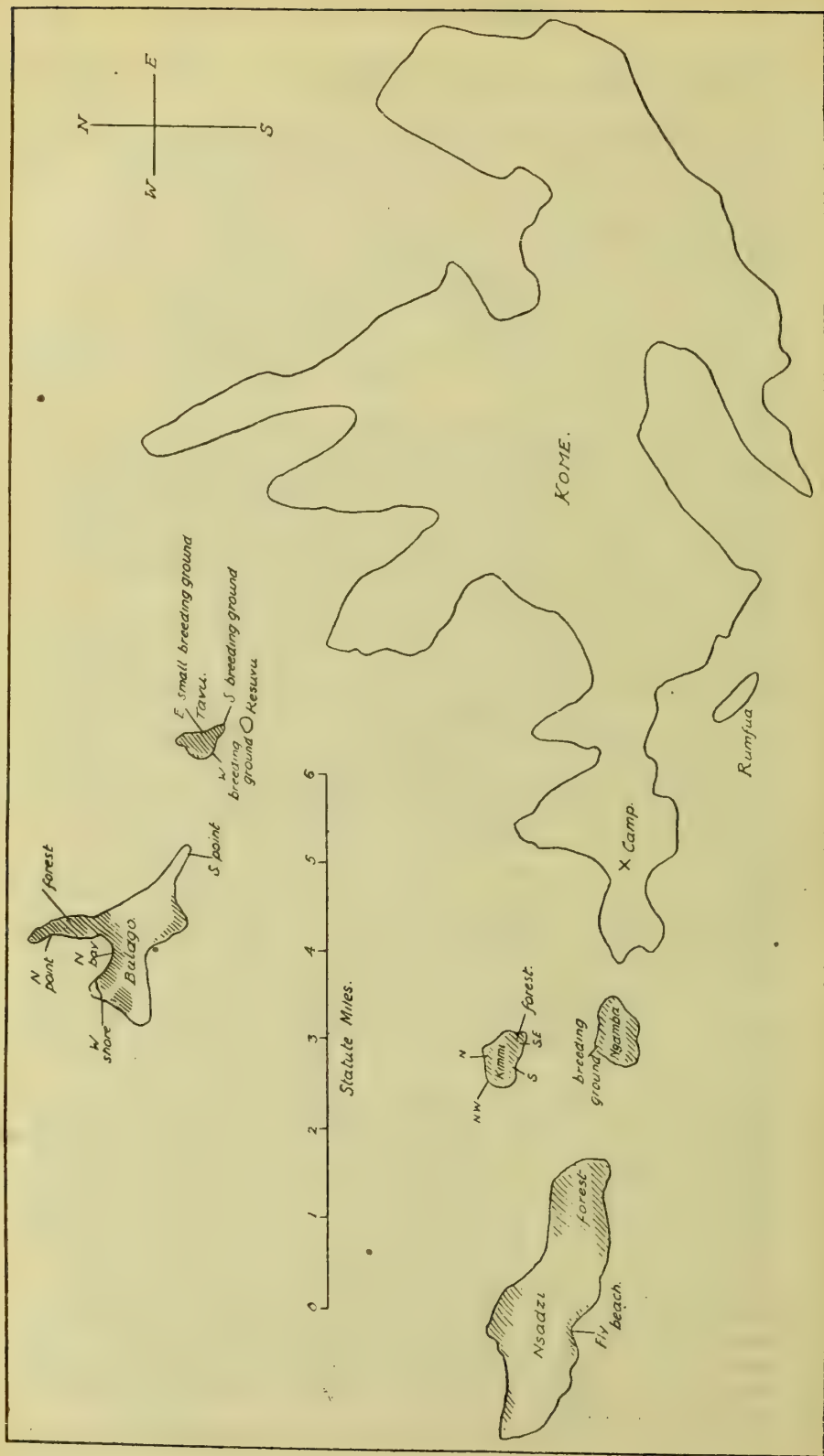
On Kimmi it was noted that the catches from the two localities on the N. and N.W. shores of the island, taken together, varied *inversely* to the catches from the S. and S.E. shores, taken with the forest locality at the S.E. part of the island (*see Chart 1, p. 77*). This, however, was not so marked after the beginning of July. This inverse relation means that at certain times the ♂ flies find the N. part more attractive or more congenial than the S. part, and vice versa. I could see no variation in the food supply that would account for it.

It was stated in the last report that "there appears to be some grounds for believing that the accessibility of reptilian food supply is one of the factors influencing the numbers of *G. palpalis* on the islands."

Kimmi was taken as an example. When visited early in 1914 there was a very much higher percentage of female flies than had been met with anywhere else, and they were obviously extremely hungry. It was concluded that "if the explanation of the high ♀ %age be scarcity of food supply, and if this reacts unfavourably on the rate of reproduction, it cannot be expected that the flies should remain in their present condition of great numbers with high ♀ %age. The numbers at least must decline, unless the food supply becomes increased, in which case the ♀ %age must fall." It may be said at once that the number of females *did* fall in a most remarkable manner in March; but rose again in April, though not to the former height, and then remained low, only just higher than figures for the other islands (*see Chart 2, p. 78*).

The ♂ per boy hour figure, however, which indicates the actual

MAP II.



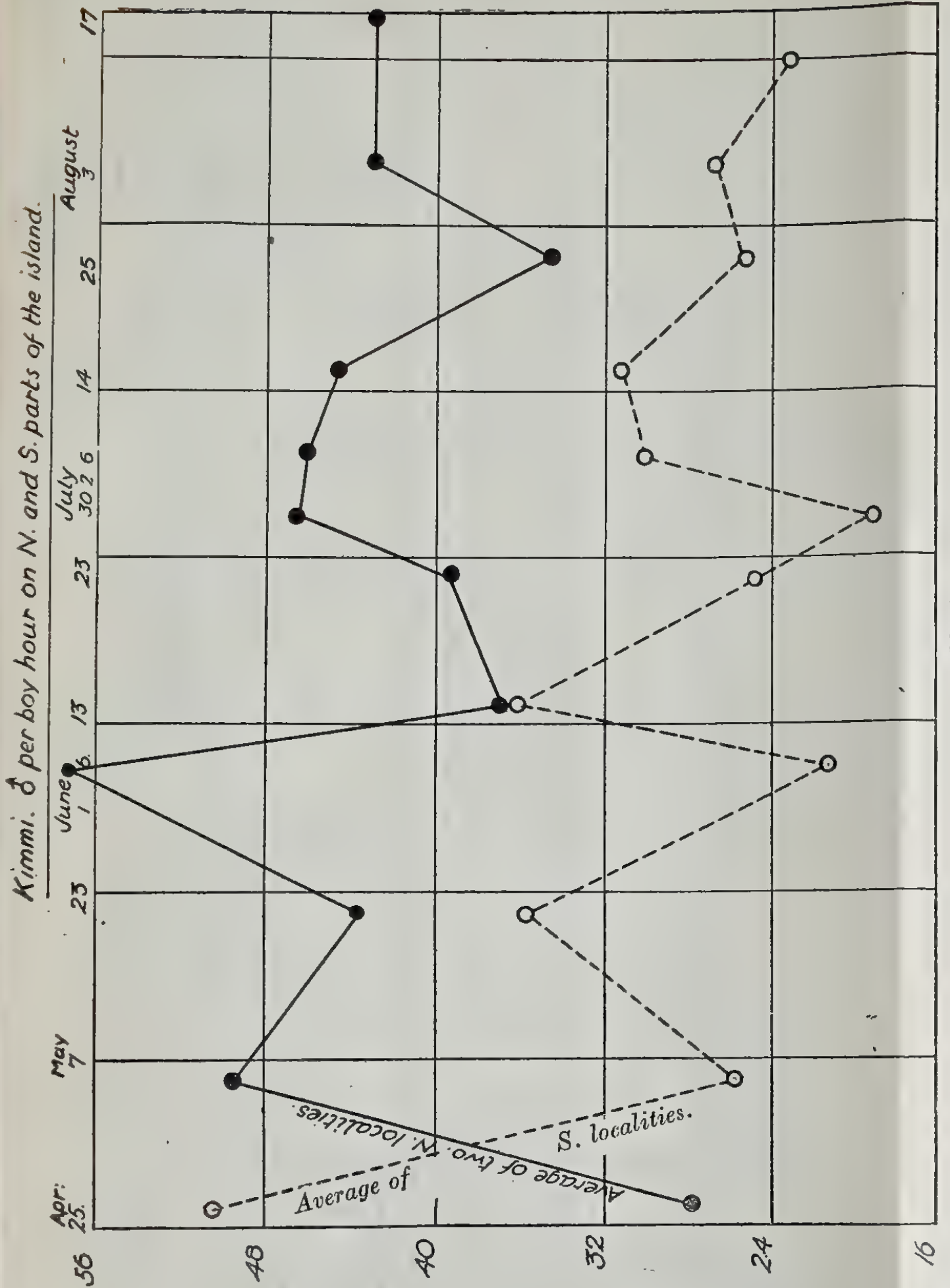
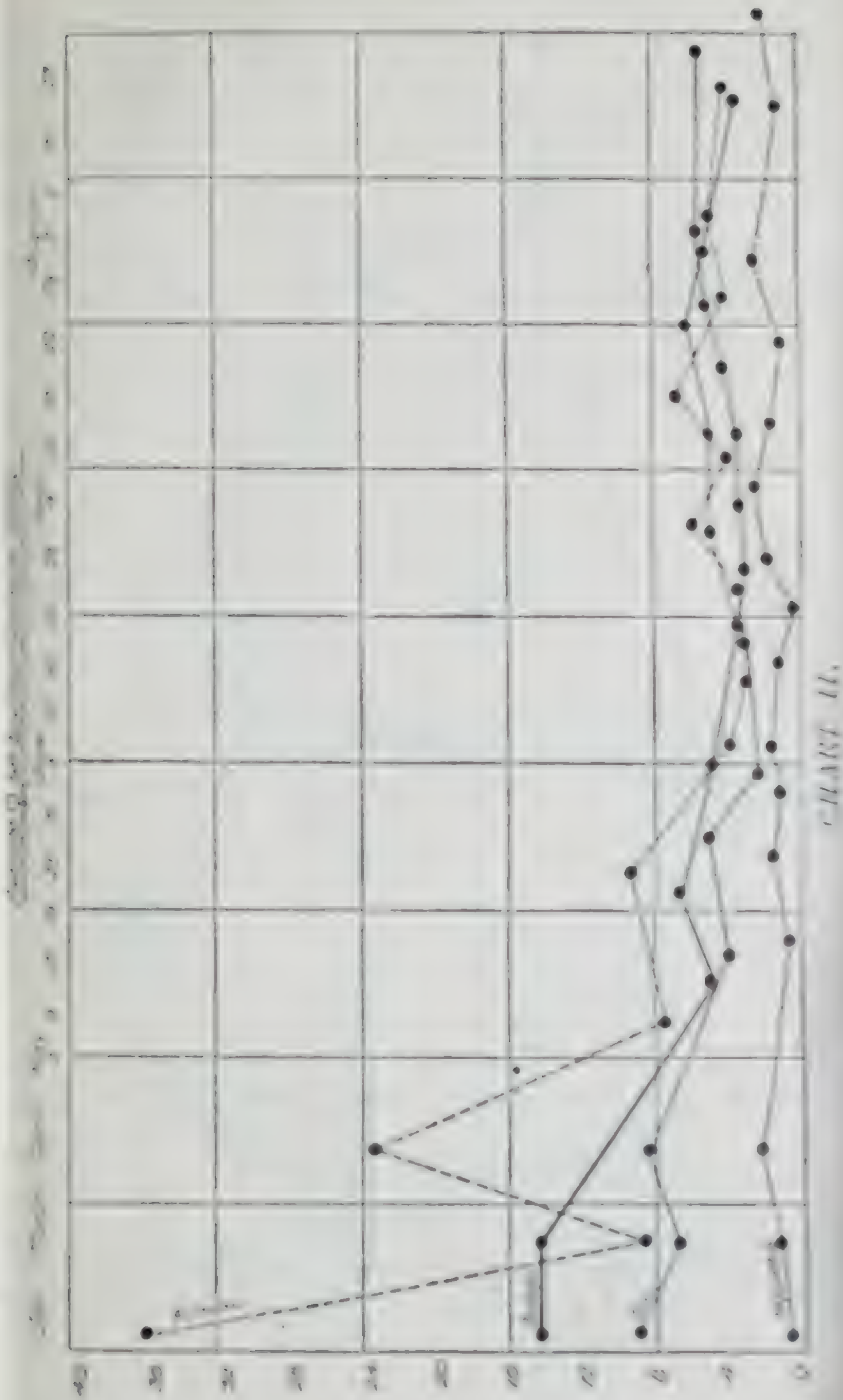


CHART I.



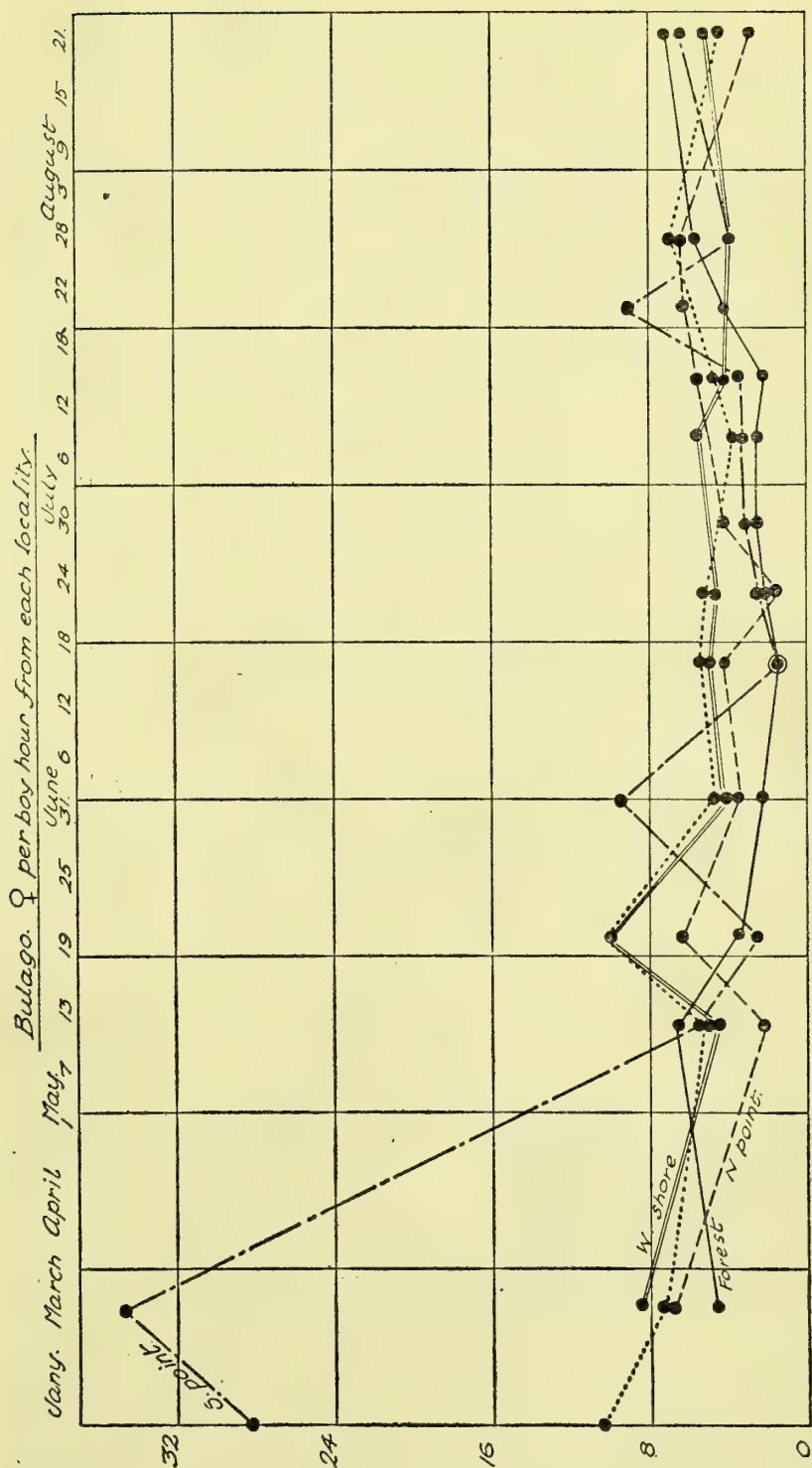


CHART V.

Tavu. Number of Crocodiles seen and ♂ flies per boy hour, (average of 3 localities)

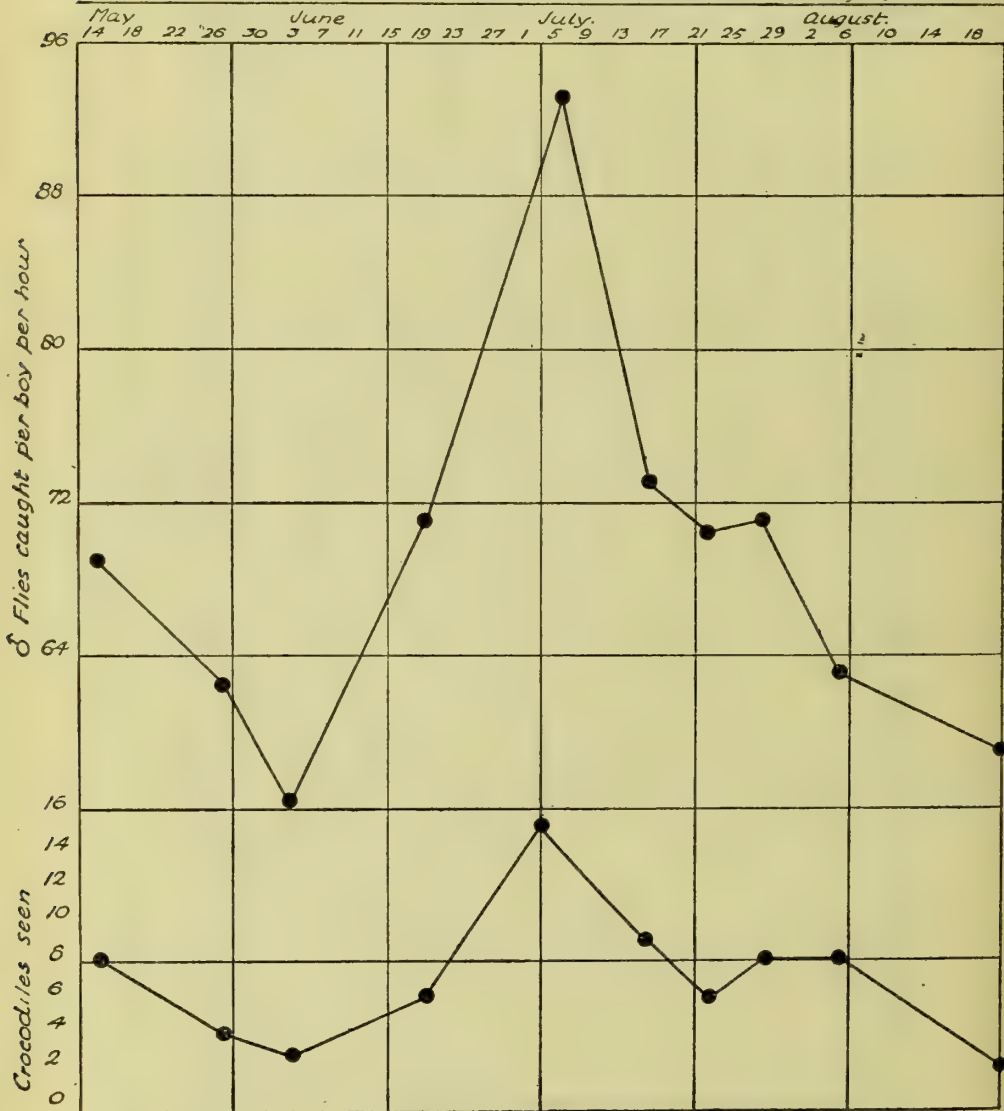


CHART VI.

number of flies present, showed no great variation, and remained at about the same level (*see Chart 3, p. 79*).

As regards the food supply, no important changes could be seen between the end of April and the middle of August, but it is possible that there had been a change from January to March, for the visit in March was not long enough for any change to be noted.

Between April and August one or more *Varanus* lizards were seen at every visit, crocodiles very often, and two nests of eggs were found on different occasions, and a python was seen, with flies feeding on it. There were always hippos in the vicinity, and their sleeping places in the forest showed that they habitually frequented the island; these, however, are of little importance as food, as is shown elsewhere in this report. So that, as regards Kimmi, it is not yet proved that alterations in the ♀ %age agree with variations in the food supply.

If the figures representing the average infestation for each of the islands be charted together, a remarkable fact becomes apparent. On the island where the ♂ curve shows the *greatest* variation, the ♀ curve shows the least, and vice versa. Thus, on Tavu, the ♂ curve shows great variation, but on Kimmi, little by comparison (*see Chart 3*); whereas the reverse is the case with the ♀ curves (*see Chart 2*). The same was found to hold good for separate localities on an individual island. Thus, on Bulago, the catches from the forest on the N. point showed the greatest range of variation in the ♂♂, but the ♀ curve was more constant than in any other part of Bulago. Conversely, the sandy spit at the S.E. end of the island had the most variable female catches, while the male catches showed less range of variation than elsewhere (*Charts 4 and 5*). In this case the variations in number of females seem to agree with variations in food supply, so far as that could be determined. For the forest and the shore on the north point had a more constant food supply, as there always seemed to be *Varanus* lizards there, whereas on the south point they, and crocodiles, came and went. This is shown below, but is perhaps not very conclusive.

Date.	Forest and N. point.	South point.
June 4..	1 <i>Varanus</i> seen	No tracks of <i>Varanus</i> or Crocodile
„ 18..	2 <i>Varanus</i> seen	1 <i>Varanus</i> .
„ 25..	None seen	No tracks of <i>Varanus</i> or Crocodile.
July 2..	1 <i>Varanus</i> seen	1 Crocodile "form," tracks of Var.
„ 9..	Fresh tracks of <i>Varanus</i>	No tracks of <i>Varanus</i> or Crocodile
„ 17..	1 <i>Varanus</i> seen	1 Crocodile, 1 <i>Varanus</i> seen
„ 24..	1 Crocodile, nest of fresh eggs, 1 <i>Varanus</i>	2 nests of fresh Crocodile eggs and 1 Crocodile seen.
Aug. 1..	1 <i>Varanus</i> seen	1 Crocodile seen
„ 20..	1 Crocodile's nest of fresh eggs, 1 <i>Varanus</i> seen	2 nests of fresh Crocodiles' eggs
„ 21..	1 <i>Varanus</i> in forest, 2 <i>Varanus</i> N. point	1 Crocodile seen

Applying the same argument to the difference between Tavu and Kimmi it must be supposed that the females always find sufficient food on Tavu, so that they do not come to be caught,

whereas on Kimmi there must be a less constant supply, which causes females at times to be more hungry than at others.

In this connection another very interesting fact has become apparent. Tavu Island was much frequented by crocodiles, and every time it was visited it was first circumnavigated, and the number of crocodiles seen on the shore or in the water just off the shore was counted, the maximum ever seen was 15. Part of the attraction, no doubt, was the sandy point at the south of the island, which was eminently suitable for their nests, of which several were found from time to time. This seems a convenient place again to allude to the relation between *Varanus* and crocodiles.

Every nest of crocodile's eggs that I found on the four islands (10 altogether) has sooner or later been rifled by *Varanus*, which must be continually increasing in number now that they are no longer kept down by the hunting of natives. So that it seems that as they increase, crocodiles must decrease.

The number of ♂ flies that was caught on Tavu was always high and very variable, and it was found that the variations coincided very closely indeed with the number of crocodiles present on the island at any visit; the more crocodiles, the more flies, and vice versa (see Chart 6). The female flies, however, showed no such relation, the variation being very small.

Tavu has very few *Varanus* in comparison with the other islands, though there were always enough to rifle the crocodiles' nests. So the conditions on Tavu and Kimmi are in some respects complementary; on the former a very variable, though always abundant, supply of reptilian food, and the number of male flies varying concurrently; on Kimmi a less ample, but perhaps more constant supply and the male flies much less variable; on Tavu the female flies caught in fewer numbers, but not very variable; on Kimmi more females caught, with greater variations.

The relation between crocodiles and male flies on Tavu seems to me difficult to explain. For it cannot be supposed that, on a day when many crocodiles are lying on the shore of Tavu, other flies are attracted to the island from Bulago or Kome, which are separated by a considerable space of water (see map, p. 76).

If it were on a part of the mainland shore that one found a number of crocodiles associated with a number of flies it would be natural to suppose that the congregation was due to flies that were wandering along the shore remaining in a place where food was very plentiful. It may be that the naturally more roving disposition of the male renders it liable to be caught whether it comes for food or not, so that when there are crocodiles about more are caught beyond what would have been caught in any case. Possibly they may lurk in the central forest which covers the island (the catches were made on the shore, outside the forest), and periodically fly out around the shore in search of food. If there are many crocodiles on the shore possibly the more active and enterprising male flies remain in the neighbourhood, so that more would be caught when there are more crocodiles, while the females may return to the forest again, and having fed would not come to be caught.

On Kimmi, on the other hand, where crocodiles do not congregate in such numbers, the males are not collected together, and are caught as usual whether they come to the fly-boys for food or just out of curiosity, and so are more constant, while the hungry females come at certain times in greater numbers. On the sup-

position that flies lurk in the forest, from which they make excursions in search of food, one would expect the number caught in forest localities to be comparatively constant.

On Bulago, however, where there is an excellent forest locality on the N. point, the number of flies was extremely variable, as has been pointed out earlier. The explanation of the variations discussed above must thus still be considered uncertain.

HOW LONG CAN *G. PALPALIS* LIVE UNDER STARVATION?

If it is desired to experiment upon *Glossina* by driving away its (mammalian) food supply, it is of value to know how long the fly can exist without blood food.

EXPERIMENT I.

On April 28th, 1914, two cages of freshly caught flies were taken and a ripe banana put into each. They were kept in a shaded place in the hut, but cage A got more light than B, so that the flies were more active in it.

Cage A.—Flies all dead by the morning of May 4th, but one was alive on evening of the 3rd (6 days).

Cage B.—The last fly did not die until May 7th (9 days).

EXPERIMENT II.

As it was possible that the banana might keep flies alive by virtue of the moisture given out by it into the atmosphere, flies were put into one cage and a banana into another, which was put on top of the first, the gauze sides adjacent, but so that the flies could not reach the banana, although it would have the same effect as if in the cage with the flies. The figures in the columns below indicate the number of flies found dead in the cages on any date; they were put in on May 7th.

No. of cage ..	Controls.		Banana within reach of fly.		Banana out of reach of fly.	
	1	2	3	4	5	6
Sex	♂	♀	♂	♀	♂	♀
No. of flies ..	16	14	14	8	14	7
May 9th ..	1	4	3	1	3	
" 10th ..	2	1	1			
" 11th ..	1	3	4	2	2	1
" 12th ..	4	2	2	3	4	1
" 13th ..	4	1	1	1	2	2
" 14th ..	1		3	1	3	2
" 15th ..	3	2				1
" 17th ..		1				
	16	14	14	8	14	7

According to this experiment the presence or absence of banana does not make appreciable difference. A ♀ fly in the control cage lived longest, viz., 9 days, after being put in the cage.

EXPERIMENT III. *How long can flies live after a single meal?*

On May 26th a cage of 144 flies was put on a monkey.

Day of expt.	No. of flies found dead.	
	♂	♀
3	22	
4	7	2
6	14	3
7	19	2
8	17	
9	16	1
10	15	1
11	8	3
13	8	1
14	3	
15	1	
16	1	
	131	13

Conclusion.

A fly may live for 15 days on a single feed. Experiments similar to above were made with newly hatched flies.

Date of emergence.	Sex.	How treated.	Last date on which seen alive.	Length of life.
June 26 ..	♂	In darkened cage	July 5	10
" 27 ..	♂	" " " " " "	" 6	10
" 29 ..	♂	In glass topped pill box	" 5	7
" 30 ..	♂	" " " " " "	" 8	9
July 1 ..	♂	" " " " " "	" 11	11
" 1 ..	♂	In cage with banana	" 4	4
" 1 ..	♂	" " " " " "	" 8	8
" 1 ..	♂	" " " " " "	" 6	6
" 2 ..	♂	In cage	" 7	7
" 2 ..	♂	" " " " " "	" 7	7
" 2 ..	♂	" " " " " "	" 9	8
" 3 ..	♂	In cage with piece of pineapple ..	" 9	7
" 3 ..	♂	" " " " " "	" 10	8
" 3 ..	♂	" " " " " "	" 10	8
" 3 ..	♂	" " " " " "	" 12	10
" 3 ..	♂	" " " " " "	" 12	10
" 3 ..	♂	" " " " " "	" 12	10
" 3 ..	♂	" " " " " "	" 10	8
" 4 ..	♂	In cage with capsule of water ..	" 11	8
" 5 ..	♂	In cage	" 11	7
" 5 ..	♂	" " " " " "	" 14	10
" 5 ..	♂	" " " " " "	" 14	10
" 6 ..	♂	In cage. Fed on goat once (July 7) ..	" 12	12
" 7 ..	♂	In glass topped pill box, fed on cock once	" 17	11
" 7 ..	♂	" " " " " "	" 16	10
" 8 ..	♂	In dark pill box	" 18	11
" 8 ..	♂	" " " " " "	" 16	9

Conclusion.

The longest time a newly emerged fly has been kept without food is 11 days, the average time, for 22 flies, 8.3 days. It does not seem to make any difference if fruit is available or not.

After a single feed a captured fly has been kept alive 15 days.

DOES GLOSSINA PALPALIS CIRCULATE ROUND AN ISLAND?

The question whether the fly travels round an island is of some importance in connection with attempts to destroy them by artificial breeding grounds.

It is obvious that unless the flies roam about they must make the best of whatever sites for larviposition they can find in their neighbourhood, and if there is no good breeding ground there their numbers may be kept down by the death of pupæ which are perforce deposited in unfavourable places. This is probably the explanation of the comparative scarcity of flies on islands lacking good breeding grounds.

If, on the other hand, they roam about in search of a breeding ground, an artificial breeding ground, if sufficiently attractive, is much more likely to be efficacious. For instance, on Ngamba, the one shelter that was constructed would serve for the whole island, whereas, if the female fly does not roam, several would have to be constructed on different parts of the island.

The question may also be considered in connection with the food supply. It is reasonable to suppose that a single crocodile lying on the beach would feed a great many more flies if they circulate round the island than if each was restricted in its range and was dependent upon the food supply in its own area.

An experiment was therefore made on the S. shore of Nsadzi Island which is particularly well suited for this. There is, at about the middle of its south shore, a stretch of sandy beach, perhaps half a mile long, backed by forest, forming a typical fly beach. A few hundred yards to the west of this is a patch of bush shelter, but beyond this the coast is extremely inhospitable for fly, being bare and rocky, with only low aromatic herbs and sparse low-growing thorny acacia plants. About one hundred yards to the west of the patch above mentioned is a smaller patch about 30 yards square, then open shore with no shelter for about a hundred yards, and another smaller patch of a few bushes, another stretch of bare, open coast, another clump of a few bushes, then several more hundred yards of open coast, leading on to a narrow fringe of bush around the exposed western end of the island.

It is obvious that such a coast line is not favourable to fly, and yet if one goes down from the bare grassy hill in the centre of the island to one of the stretches of open, shadeless shore between the patches of bush, one or two flies at once make their appearance, and may be seen sitting on the hot rocks in the full glare of the sun, or else they attack one viciously.

It is almost certain that these must be flies which have left the thick shade of the fly beach, or the forest at the E. end of the island about two miles away, and are circulating around the coast in the search for food and breeding grounds.

This can easily be tested by taking up a position in one of the very isolated, small clumps of bush and catching flies continuously for a prolonged period.

Accordingly, on June 10th, 1914, I put 10 fly boys in the clump that has been described as about 30 yards square, and they caught flies continuously from 9.45—1 and from 1.45—2.45; the time from 1.10—1.40 being occupied in looking for pupæ.

Time.	No. of flies caught.		♂ b.h.	♀ b.h.	Total b.h.	♀ %age.
	♂	♀				
9.45—11.15	51	18	3.4	1.2	4.6	26
11.20—11.50	16	24	3.2	4.8	8.0	60
12—12.30 ..	12	15	2.4	3.	5.4	55.5
12.30—1 ..	16	6	3.2	1.2	4.4	27
1.10—1.40 ..	One empty puparium found.					
1.45—2.15 ..	17	24	3.4	4.8	8.2	58.5
2.15—2.45 ..	9	17	1.8	3.4	5.2	65
	121	104				

For the following reasons this experiment is held to show that the flies do wander along the shore of the island, where they could not live permanently.

1. If the 225 flies had all been in the patch of bush at the commencement the first catch of $1\frac{1}{2}$ hours would have been bigger and there would have been more difference in the next.
2. The manner in which the percentage of females oscillated. Had the flies that were caught been inhabitants of that localised area only, there should have been a continual steady rise in the ♀ %age, as was pointed out in the last report, owing to the fact that the more active and curious males are first caught, so that later catches have a proportionately greater number of females.
3. The fact that after an interval while the boys were hunting for pupæ, there was a decided increase in the number caught, suggesting that more flies had entered the patch of shelter.

Conclusion.

Fly may, from an area of good shelter as a base, roam along parts of the shore where they could not reside owing to lack of shelter; presumably they are flying along the shore in search of food and breeding grounds. So that the clearing of one small part of a fly area will not necessarily prevent flies at least visiting that part if there is shelter elsewhere to which they can retire.

THE STAGES OF DEVELOPMENT OF GLOSSINA PUPÆ.

It was pointed out in the last report that interesting conclusions can be drawn from the stage of development of pupæ that have been found, and that a high proportion of pupæ in the early, creamy stage represents active reproduction, and so forth. But it was not known how long was required for the various stages. Accordingly, pupæ were collected from the artificial breeding ground on Tavu Island on July 10th. This had been thoroughly searched on July 1st, so that the 190 pupæ obtained had been, presumably, mostly deposited between July 1—10.

A few, however, had been overlooked, as shown by emergence of some flies shortly after the pupæ had been collected. Some of the pupæ were examined at weekly intervals, with the following result:—

Date.		No. examined.	Creamy.	Eyes pink.	Colour of adult.	Ready to emerge.
			%	%	%	%
July 17	..	20	95	5		
„ 25	..	20	75	20	5	
„ 31	..	20	35	55	5	
Aug. 5	..	10		80	20	
„ 17	..	10			90	10

Between 16th and 24th August inclusive 8 ♂ and 8 ♀ emerged. Between July 13th and August 1st, 4 ♂ and 5 ♀ emerged; these pupæ had almost certainly been overlooked when the ground was searched on July 1st, and had been deposited before that date, so that when found on July 10th they had already gone through a certain stage of development.

It is seen that about 26-36 days are required for the majority to reach the stage when the eyes get darkened with pigment, the limbs being outlined but the body wholly white. By the 38-48 day the majority show the colours of the adult, and in about a week more the flies emerged.

Eighty-five of the pupæ died, but the remainder emerged between August 16th and 24th.

G. D. HALE CARPENTER.

September 14th, 1915.

MEMORANDUM ON A PROPOSED NEW METHOD OF ATTACKING GLOSSINA PALPALIS.

BEING A FIFTH REPORT ON ITS BIONOMICS.

Methods of attacking obnoxious or destructive insects depend for their efficiency upon the discovery of some vulnerable period in the life history of such insects, during which they may be conveniently or easily destroyed.

It is therefore obvious that the first step in success is the discovery of such a vulnerable point, and for that purpose investigations of a thorough nature, observational and experimental, require to be carried out, possibly for prolonged periods.

The life history of a very large number of insects is characterized by alternate stages of quiescence and activity; this is the case with all such as undergo complete metamorphosis, that is, that pass through four stages of egg, actively feeding and growing larvæ, quiescent pupa, and adult.

Insects such as locusts, scale-insects, and others of economic importance in which the metamorphosis is incomplete, need not be considered here.

In the case of the *Egg* stage, the female of certain destructive species of moths deposits all the eggs together in a mass made more conspicuous by tufts of hair which she takes from her own body and places as a covering over the eggs. Such masses are easily found on leaves and twigs and destroyed.

The next stage, the *Larva*, offers facilities according to its habits: the familiar example of the method of oiling water so as to drown mosquito larvæ scarcely needs quoting.

The quiescent *Pupal* stage is not quite so easily dealt with. The places whither the larva retires in order to pupate require to be known for each species; the pupæ may be searched for by digging around the roots of the food plant, or may be destroyed by collecting and burning rubbish among which it makes its cocoon, etc.

The adult insect, or *Imago*, with its infinite variety of habits, is vulnerable in proportion to our knowledge of such habits and the opportunities they afford.

A good example is the destructive winter moth of England, whose wingless female, having emerged from the pupa in the ground, climbs up tree trunks in order to lay her eggs on the leaf buds. The method of attack based on this is to affix around the trunks rings treated with appropriate material which prevents the passage of the crawling moth.

Another striking example of what may be accomplished by application of a thorough knowledge of a harmful insect is a method of attacking a Fruit Fly. It was noted that the female emits a certain odour, presumably to attract the male. It was found possible to reproduce the chemical compound responsible for the odour, and to embody it in adhesive material spread on fly-papers. Very large numbers of the male flies, attracted by the odour, could thus be destroyed.

It may here be remarked that a method directed against the *female* sex is of more value than one against the male, seeing that one male insect will suffice for a number of females, and this is of significance in connection with the method about to be described.

The above principle must be considered thoroughly well established, and it remains to be asked how it can be applied against *Glossina*. A consideration of its highly specialized life history, almost unique among insects, shows that nothing can be done to destroy the egg or larva.

For an egg never passes from the body of the mother fly, wherein it develops to the larva which is nourished in the fly's abdomen until it is fully fed. The larva is then extruded, in a *suitable locality*, burrows a little way, and becomes the inert pupa from which the active, roaming fly ultimately emerges.

What, then, are the prospects of successful attack in the only two stages available to us? In the case of the adult fly a good deal may be done, but the expense and labour and risk involved are considerable. It has been amply established that *G. palpalis* is absolutely dependent upon the shade (of bushes mainly) and cannot live in open country.

Hence clearing away the low bush is known to drive the fly away, but this method is impracticable over such an enormous area as the shores of L. Victoria.

For small areas, however, such a method is practicable, and combined with systematic catching of the fly has freed the valuable islands of St. Thomè and Principe, on the West coast, from *Glossina* which was seriously interfering with the cocoa trade.

We now come to consider the point to which this memorandum is especially directed, namely: Is there no means of attacking *G. palpalis* in its *pupal* stage?

I propose to show that there is prospect of success by adopting

a method founded on intimate knowledge of the requirements of the pupa. It was said, a few paragraphs back, "the larva is extruded in a *suitable locality*," and these underlined words contain the whole kernel of the matter.

What, then, are the requirements of the pupa? During the latter part of a period of over 40 months spent in observational and experimental work on *L. Victoria* I have particularly directed attention to this point, which was treated at length in my *Third Report*, written for the Royal Society in 1914, but not yet published owing to the war.

The following are the main points (*vide* above report): *The pupa requires loose, dry soil, well shaded, but with the surface thoroughly ventilated; within a few yards of the water, but beyond its reach.*

Such requirements are satisfied over large areas by the raised beaches left by subsidence of the lake from its former higher level, and now overgrown by bushes and other vegetation. These areas are termed *Breeding grounds*.

These requirements may now be considered *seriatim*.

I. THE SOIL.

It is obvious that this must be *loose*, else the larva cannot burrow into it. Hence, however suitable a place may be in other ways, if the soil is closely bound together by roots the place is not suitable as a breeding ground.

The character of the soil forming a breeding ground may be any of the following, which have all been found to contain pupæ in varying numbers on the islands indicated in brackets.

- A. Fine pebbles. (Yempata.)
- B. Pebbles mixed with coarse sand. (Wema, Damba, Tavu.) This appears to be the most suitable medium.
- C. Coarse brown sand. (Kerenge, Sindiro, Bulago, N. Nsadzi.)
- D. Coarse white sand. (Bulago, Kimmi.)
- E. Very fine white sand. (Bugalla, Yempata, S. Nsadzi.)
Often holds too much moisture to be very favourable.
- F. Light friable earth. (Kerenge, Ziro, Bulago.)
- G. Vegetable humus and débris. (Lula.)
- H. Very fine dry dust, as found in caves (Kimmi and Waitwe), mainly derived from the desiccated and disintegrated droppings of bats. This never provides many pupæ.

Heading G. is of great importance.

This material does not occur over large areas in suitable condition to form large breeding grounds, but over many a long stretch of rocky shore little pockets of it under shelter of rocks or fallen tree trunks often form the only suitable sites for deposition of larvæ. Since the term breeding ground would not differentiate such places from the areas previously described, I propose the word *Locus* for such a place. It will be shown that such *Loci* can sustain *Glossina* in abundance in complete absence of breeding grounds.

- II. The soil must be *dry*. Pupæ are never found in damp environment: rotting dead leaves are inimical to them.

III. The place must *be well shaded*; the shade from high trees alone is not sufficient; pupæ require shelter nearer the ground. Such shade may be permanent or variable. *Permanent shade* is afforded by:—

- A. Caves, or large under-cut rocks (Kimmi, Wema, Damba).
- B. Prostrate tree trunks arching over the ground, as figured in my first report. (Report of S.S. Commission of Royal Society, No. XII., p. 97.)
- C. Hollows at the base of or among roots of living trees, as figured in my second report. (S.S. Commission, Vol. XIV., p. 35.)
- D. Thick bushes, as figured in first report. (Loc. cit., p. 96.)

Variable Shade.

This is of importance from several points of view.

Sundry straggling creepers, and a low-growing aromatic herb, common on the lake shore, at times become sufficiently dense to throw good protective shade, and such Loci are immediately seized upon by the fly. Subsequently, when for some reason or another the shade is thinner, the pupæ in the ground at the time are killed and no more larvæ are deposited there by the fly.

The following observations may be cited from my third report:—

Obs. 1. On Wema, in 1914, a very thick tangle of young green shoots of a species of *Polygonaceæ* was found growing on a ridge forming part of a fly-beach, so as to arch over the surface below.

One boy found here 88 pupæ, of which 91 per cent. were found on examination to have been recently developed from the larva. Moreover only six empty shells, from which the adult fly had emerged, were found. That is to say, only very recently had the young shoots begun to throw shade thick enough to cause the fly to deposit larvæ there.

Obs. 2. Contrast this condition with the following.

On Bulago a boy searched for pupæ at the base of a small papilionaceous tree with thin foliage that even at its best throws poor shade. This tree had recently died and the rambling plants around its base which had evidently at one time made good shade, were thin and poor.

There were found 132 shells from which the fly had emerged, and five pupæ, all dead. It was obvious that at one time the herbs had been dense and green, and the fly had found the spot suitable for depositing larvæ but that it had ceased to do so.

Obs. 3. The following example is most instructive.

On Bulago was a tree throwing out large horizontal branches near the ground, arching over which was a canopy of half-dead vegetation, throwing, at the time, poor shade, but with some fresh green shoots springing out.

Forty-five pupæ were found here, of which 27 were dead; in 3 the adult fly was almost ready to hatch, and the other 15 were in the earliest stage of development.

The facts may be thus interpreted. The herbage at one time had been dense enough to throw good shade, but, having died, many pupæ beneath it were killed by exposure. Now, however, that fresh young shoots were re-spouting, the fly was again using the spot as a breeding ground.

IV. The surface of the soil must be *well ventilated*.

A collection of mouldy, dead, leaves and sticks on top of the soil renders it unfavourable, and however well suited the other conditions may be the fly will not deposit larvæ there.

Obs. 4. A very striking instance of this, on Tavu, is given in my third report. Pupæ had been searched for unsuccessfully under the shelter of some dense bushes growing on a raised beach, where the conditions seemed favourable, but the surface was covered with a layer of mouldy, dead leaves.

A fortnight later I re-examined the little area of about two square feet from which the dead leaves had been removed by the former search, so that the sand had been left exposed. On this occasion, in a few minutes, *two dozen* freshly formed pupæ were found.

V. Pupæ require to be *near the water*, within 20—30 yards.

This fact could never be proved until a recent observation made it clear. For the argument could always be put forward that it is only near the water that the shaded, raised beaches, which provide the optimum conditions, are found.

The consequences of the rise of the lake level in 1917 to a height not attained since 1906 have, however, afforded the proof required.

Obs. 5. On the S.E. coast of Kimmi, in 1914, was a broad, flat strip of low-lying land, covered with lush green grass and other vegetation, soft and waterlogged, much frequented by hippos. and known for reference as a "hippo. grazing ground."

This obviously owed its origin to the level of the lake having fallen since 1906, so that the water had receded from the real shore, marked, at the edge of the forest which covers that part of the island, by the usual raised beach which was thus separated from the water by a hundred yards of marshy grassland. This raised beach satisfied all the requirements of pupæ except proximity to water, but in 1914 none were found there. In December, 1918, the water was found to have come back over the grazing ground up to the former shore at the forest edge, which thus formed a typical "fly-beach," and *was used by the fly as a breeding ground*, for numbers of pupæ were found there. The same sequence of events, to a less noteworthy degree, was found on the N. shore of the island.

The above five conditions together comprise the requirements of a good breeding ground, and the conclusion stated in my third report may be again affirmed.

"The fly can exist in certain numbers in localities where there are no breeding grounds; for greater abundance breeding grounds are necessary."

This conclusion was based on a comparative study of a large number of small islands in January—March, 1914. Certain of

these, *e.g.*, Lula and Kiuwa were found to be without any areas characterized by the five conditions established above, and yet on Kiuwa the fly existed in numbers represented by a catch of 15 males per boy per hour.*

On Lula, in December, 1918, the number of fly is represented by the astonishing figure of 56.5. This is a strip of rock some 60—100 yards long by 20 broad, composed entirely of piled up rocks covered with ferns and bushes, furnishing abundant loci, but no breeding ground.

This figure, however, is exceptional. An investigation of some miles of shore along N., S., and E. coasts of the large island of Kome, where breeding grounds are few and far between, would put the average figure for fly in the absence of breeding grounds as about 25—30.

That the loci upon which the fly depends in such areas are very scattered is shown by the fact that Mr. Fiske, I, and a dozen fly-boys, making a systematic search for two days on Kiuwa, only found pupæ at a rate of 3.3 per boy hour.

These facts show the *futility of any attempt to destroy the fly by merely clearing the shelter on ascertained breeding grounds.*

It is of some interest to know to what figure the average catch of males per boy per hour may attain. The following is of value in this connection.

Obs. 6. A long stretch of at least five miles of the northern coast of the western end of Kome was examined for fly and breeding grounds, landings being made from the canoe when suitable localities for catching fly, that might produce pupæ, showed themselves.

Locality.	♂ ♂ per boy hour.	Pupæ per boy hour.
<i>a</i>	27	Not searched for—no breeding ground
<i>b</i>	76	?
<i>c</i>	38	Not searched for—no breeding ground.
<i>d</i>	54	20.5
<i>e</i>	11	Three boys found 2 pupæ in $\frac{1}{2}$ hour.
<i>f</i>	35	Three boys found no pupæ in $\frac{1}{2}$ hour.
<i>g</i>	38	" " " "
<i>h</i>	125	132.5
<i>j</i>	35	One boy found 78 pupæ in $\frac{1}{2}$ hour.
<i>k</i>	16	One boy found one pupa in an hour.

The whole of the shore of the two western bays on the north of Kome was thus inspected (*see map, p. 76*); its greater part has forest up to the edge of the water, the shore is either of rock shelving gradually, or broken into piled up boulders, or fringed with papyrus and reeds. No "fly-beach" is found, but here and there small collections of sand or gravel may be found and these are used as breeding grounds.

Where these occurred the numbers of fly were correspondingly increased; this was the case at loc. d and h.

Loc. d, being of interest from another point of view, will be considered later.

*Note.—Mr. Fiske showed in 1913 that the only reliable standard of comparison is this method of estimation. The females that are caught vary enormously in number according to their state of hunger.

Loc. h consisted of a very small recessed bay, some 40 yards across at the water's edge, filled with sand, sloping back from the water to a distance of 20 yards. On this sand some bushes provided good shelter and furnished pupæ at a rate which is near the record. The catch of fly in that locality reached the record, 125 per boy hour (based on catches of four boys for half-an-hour).

Loc. b must certainly have had a breeding ground in the vicinity but search was not made at that spot as there seemed at first sight to be nowhere that could serve.

Loc. j is of interest. The shore was of flat, shelving rock, but at one point a little gravel was sheltered by a tress of hanging creeper just touching the ground. At this point one boy found 78 pupæ in about a square foot, in half-an-hour.

Obs. 6. Again, similar examination of some three miles on the east end of Kome has given the following figures.

Locality	♂ ♂ per boy hour	Pupæ.
k	26.5	None found.
l	27	"
m	34.5	One found by 4 boys in $\frac{1}{2}$ hour.
n	15.5	—

Nowhere along this part of the coast was there a breeding ground. The conclusion (No. 16) in my second report may, therefore, be re-affirmed; "the prospects of getting rid of *Glossina* by limiting one's attentions to localised breeding grounds are not good."

The point to which I now wish to direct attention is the theory that the gravid female fly wanders about *searching for a suitable spot* for the young with as much care as a female butterfly employs in searching for the correct food plant.

Just as in the latter case eggs are not laid until the correct species is found, so the larva is not extruded until the fly has found the requisite conditions above described.

It is even possible that, when a fly has found a suitable spot and extruded her larva there, reproduction may be stimulated, another egg descends the oviduct, and another larva quickly nourished to full growth may be extruded in the same spot a few days later.

Hence discovery of a suitable spot by parent flies (Ref. Obs. 1—4) may very quickly increase the population at that locality.

It does not appear at all probable that the intra-uterine growth of a larva is automatic, and that it is perforce expelled from the abdomen of the parent as soon as it has attained full growth.

Such a process would result in the death of great numbers of pupæ in unsuitable environment, which should be discovered by such a systematic search as was made on Kiuwa, but were not.

Except as quoted in Obs. 1—4, such dead pupæ are not found.

What evidence is there that the fly does roam about?

In 1914 I endeavoured to prove this on the south shore of Nsadzi, and the following experiment was described in my fourth report (see p. 88, above).

There is a typical fly-beach, backed by forest, at the middle of the south shore of Nsadzi, west of which the coast for about a mile has no shelter for fly except occasional small patches of cover or an isolated bush. Flies were caught in one of these small patches about 30 yards square, by ten boys from 9.45—2.45; except from 1.10—1.40 when pupæ were searched for, only one shell being found.

Time.	No. of flies caught.		♂ b.h.	♀ b.h.	Total b.h.	♀ %age.
	♂	♀				
9.45—11.15	51	18	3.4	1.2	4.6	26
11.20—11.50	16	24	3.2	4.8	8.0	60
12—12.30 ..	12	15	2.4	3.	5.4	55.5
12.30—1 ..	16	6	3.2	1.2	4.4	27
1.10—1.40 ..	One empty puparium found.					
1.45—2.15 ..	17	24	3.4	4.8	8.2	58.5
2.15—2.45 ..	9	17	1.8	3.4	5.2	65
	121	104				

That flies were constantly entering this small patch of cover is shown by—

1. If the 225 flies had all been there at the commencement the first hour and a half's catch would have disposed of most of them instead of less than a third.
2. After a interval of half-an-hour the rate per boy hour rose, especially in the case of females.
3. The percentage of females to males did not rise steadily as it would have done in an isolated area (ref. my third report).

As a matter of fact it is obvious that the parent *must* deliberately seek out and select a suitable site. How else can be explained the concentration of pupæ at one spot as exemplified by observations quoted above (particularly locality j observation 6)?

An extremely suggestive and important observation fell to my lot on Kome in December, 1918, during the systematic search along its N. shore above described in Obs. 6.

Obs. 7. At *locality d* of that series there was an open space in the otherwise unbroken line of forest, perhaps 200 yards across and extending about half that distance back from the edge where the flat, bare rock shelved gently into the water.

This space of open grassland was surrounded on three sides by the wall of forest. But close to the water, and occupying about one-tenth of the frontage of this open space, was a very narrow ridge of coarse, brown sand and fine pebbles, on which grew a line of bushes, forming a good breeding ground.

Having found the fly to be more numerous at the borders of the forest around this open space than elsewhere in the vicinity (see Table, Obs. 6), I set the four boys to find the pupæ that produced them.

Three of the boys at once went to the ridge just described and in half-an-hour obtained 57 pupæ and 55 shells; the other boy found no pupæ but only shells (21) at the edge of the forest.

Now no fly would cross this open space except to seek food for itself or shelter for young, and this isolated breeding ground in the open seems to afford conclusive proof of careful search by a roaming fly.

From the preceding pages it is obvious that a stage has been reached when it may be asked:—

If the requirements for which the fly searches are known, and if the fly recognises certain places as eminently suitable for larviposition, *cannot some method be devised to entice her to deposit the larvae in places where they can be easily collected and afterwards destroyed?*

In other words, I suggest the *provision of attractive artificial "pupa shelters"* as a means of reducing the fly's numbers.

In localities where no breeding grounds exist and the fly is dependent upon loci, obs. 7 shows that the method is likely to be successful; the attractiveness of the isolated area described above could not be doubted, and it will be shown that artificial breeding grounds can be made attractive.

In 1914 I had already commenced experimental work on these lines (see my Fourth Report), which was unfortunately cut short by the call of active service in August. It seemed to me then that, in areas where there was a large extent of good natural breeding ground, this would require to be rendered unsuitable by clearing before the fly could be expected to use artificial shelter.

Two isles were chosen for the experiment, Tavu and Ngamba. The former abounds with fly and has two good breeding grounds (one on the west coast, one on the S. point), the former of which was cleared so that it would no longer serve, and a shelter, of the type described later, was erected on the raised beach which had formerly served as the breeding ground. Ngamba Isle has very few fly; it is almost entirely rocky but at its N.W. corner a sandy or gravelly area formed a mediocre breeding ground; this was cleared and a shelter erected as on Tavu. They were considered suitable by the fly and larvæ were deposited there.

On Ngamba the maximum number of pupæ found was represented by a figure of 9 per boy per hour, which is *more than three times* the number that had ever previously been found on that island on which, as was said, fly were few.

On Tavu the best result was obtained some two months after the erection of the shelter, when, on August 16th, 295 pupæ were found, representing a rate of 59 per boy hour. These 295 has been deposited since July 30th, when the shelter had been last cleared out.

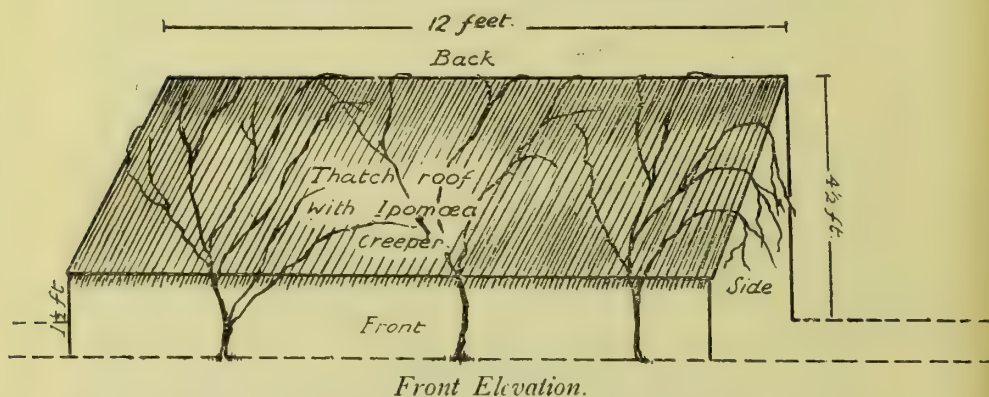
It is claimed that these results are sufficiently promising to make the method worth attention and further experimental trial for which the islands are singularly well suited.

The method being only in its infancy can doubtless be improved: the material forming the shelter, its height above ground, the size, shape, and orientation of the shelter, proximity to

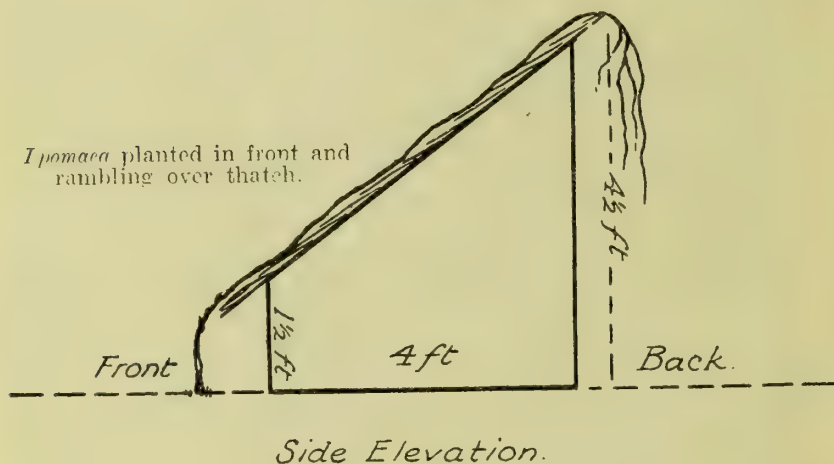
water and surrounding large trees are all points variation of one or more of which may make a pupa shelter more attractive.

Observations quoted previously suggest that the shelter formed by fresh, green growth is highly attractive, and this can easily be reproduced by planting creepers so that they ramble over the

CONSTRUCTION OF A SHELTER.



Loose sand or gravel, cleared of growth, and lightly sprinkled with leaves and bark.



Area. Twelve by four feet.

Height. At back, 4 1/2 feet.

At front, 1 1/2 feet.

Roof. Thatch, with good eaves, in front just short of the ground.

Sides. Open.

Back. Open, but protected by eaves and Ipomæa hanging down.

Uprights. Preferably metal, at least at base, owing to Termites.

Orientation. The low front should face direction of prevailing wind, and the direction from which storms come.

Ipomæa. Found everywhere on lake shore—has a purple convolvulus-flower. Native name "Aka-landalugo."

shelter. A further important point was brought out during the first use of pupa shelters in 1914.

On one occasion, on Ngamba Isle, all the pupæ that were found had been deposited at one point where a large, dead leaf had been left lying on the ground which was elsewhere bare.

Although it has been shown that a *thick* layer of dead leaves is inimical to pupæ yet it seemed possible that a light covering might be favourable and accordingly, on Tavu, for one week half the area sheltered was lightly strewn with dead dry leaves, the other half was left bare.

At the next visit pupæ were found at the rate of 51.5 per boy hour under the half with leaves, as against 34.8 in the other half.

For another week conditions were reversed, the former half being left uncovered, the latter leaf-strewn. At the next visit the leaf-strewn half again provided more pupæ, this time twice as many as the other, showing that the difference had been due to that, and no other, difference between conditions under the two halves of the shelter.

Further investigations showed that it made no difference whether leaves or flakes of bark and small logs laid on the surface. At another visit 147 pupæ were found in the bark-strewn half as against 148 in the leaf-strewn half. This condition, *of the surface lightly covered*, should, therefore, be added to the other five requirements of a good breeding ground.

The method of artificially attractive breeding grounds, or pupa shelters, as set forth obviously depends for success upon whether, in the presence of natural breeding grounds, the fly can be induced to deposit larvæ in the shelters, *i.e.*, can they be possibly more attractive than nature?

Where there are only scattered loci there is good reason to hope for success. But what would be the case on an island such as Kimmi, or a long stretch of coast such as the Buddu coast at Bukakata, where there are very large areas of natural breeding ground? Must these first be destroyed? I believe not, and the following observation on Kimmi is to the point.

Obs. 8. Mention has been made of the fly-beach and breeding ground made on that island by the return of the lake to a former higher level. Although the breeding ground as a whole appeared highly suitable, yet comparatively few pupa could be found save at one spot, three boys in half-an-hour obtaining 42, giving a rate of 28 per boy hour.

The exception exactly reproduced the conditions of my shelter on Tavu. A large tree had been uprooted by wind but had only fallen a little from the perpendicular, being supported by a neighbour. The area of earth embraced by the roots that had been torn up formed a pent-house roof at half a right angle, sheltering loose, dry soil of precisely the kind required.

In the area of a few square feet thus transformed into a small breeding ground, four boys in half-an-hour secured 309 pupæ, giving a rate of 154.5 per boy hour. That is to say, this particular spot was hyper-attractive beyond the surrounding quite suitable area, and pupæ were concentrated there to a degree never previously matched.

SOME REMARKS AND CRITICISMS.

1. This method cannot be expected absolutely to *exterminate Glossina*. But if their number can be immensely reduced their infectivity as a whole must be reduced in even greater proportion. For not every fly can obtain blood from an infective source (the Sitatunga), and if it can the host may not be at that time infective to it, as has been amply demonstrated at Mpumu.

Even were it so, of flies fed on an infective host, only a very small proportion provide suitable conditions for the ultimate development in them of the Trypanosomes they may have acquired. Moreover exactly the same factors obtain in the infection of an antelope; the chances of a single uninfected animal becoming infected and thereby acting as a permanent source of infection to flies, are also disproportionately reduced.

It must be borne in mind, however, that it will be some years before these factors, operating in a vicious circle, will have attained their full potency for good, since the very numerous Sitatunga are probably infected in a high proportion at present owing to the super-abundant fly.

It may be remarked that the presence of a few *Glossina* at Entebbe at the present day is not found to be fraught with danger.

2. The method of attacking the *adult fly* has already been shown to be practicable and leading to good results.

That is true, but for *limited areas* only, as was pointed out above. The method would be impracticable over an area such as the shores of L. Victoria.

Moreover by catching the adult fly, only a small percentage of *females* (rarely greater than 25%, often less than 10%) is destroyed, many of which will have already provided a future generation.

The method of destroying pupæ *accounts for 50% females*.

3. The breeding ground on Damba Island was used for several years as an unfailing supply of pupæ for Mpumu laboratory, thousands of pupæ being collected monthly without apparent diminution. But this was only one of many on the island. My method provides a *breeding ground for every mile of coast*, every one of which will be systematically cleared out at regular intervals.

Moreover, a breeding ground on the mainland shore of Buka Bay, at first a source of pupæ for Mpumu, was eventually given up for, as Dr. Duke informed me in 1911 (see my First Report), "there has been such a diminution of flies in this locality that it is no longer worth while to collect pupæ from there." This breeding ground, as is often the case, may have been an isolated one along a considerable stretch of shore.

4. This method demands *most regular attention* to the pupa shelter. For if a large number of pupæ collected there are allowed, owing to a visit being missed, to remain and

develop, progress in reduction of fly will be greatly hindered; indeed, the fly will be temporarily increased.

The shelters must be kept in good repair with *watertight roof*.

A regular beat could be arranged for a canoe, whereby a certain mileage of coast could be covered, and the shelters at every mile regularly examined.

Those parts of the coast fringed with a Papyrus belt might be left out of consideration—the fly only wanders into them from beyond the limits of the Papyrus.

5. This method is the simplest, least costly, and most practicable of any that has been proposed as a means of reducing *Glossina*. Being founded on natural principles it cannot be expected to have a catastrophic effect, but to produce a gradual reduction and maintain the effect so as to keep the fly at a minimal, harmless number, below which no method short of defoliating the whole country could bring it. It is at least worthy of a trial.

G. D. HALE CARPENTER,

Kome Island, January 3rd, 1919.

